Dissolved Inorganic Nitrogen Cycling in a River Receiving Wastewater: the Response to Changes in Wastewater Treatment Effluent

by

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AUTHOR'S DECLARATION

I hereby declare that I am the sole author of this thesis. This is a true copy of the thesis, including any required final revisions, as accepted by my examiners.

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Abstract

Nitrogen is an essential element for all life forms, but when in excess in an aquatic ecosystem, it can cause an imbalance in the trophic status. A significant amount of nitrogen is released into the environment by wastewater treatment plants, representing a major point source of reactive nitrogen in urban environments. This nitrogen source presents a potential threat to the ecological integrity of the aquatic ecosystem if poorly managed and monitored. Wastewater discharges can increase the primary production in surface waters, frequently degrading the integrity of the receiving aquatic ecosystem through the addition of high organic matter loads and the associated oxygen consumption during oxidation of the organic matter. The Grand River (south western Ontario) is a river highly impacted by human activities; the watershed is home to approximately 925,000 inhabitants, has approximately 34 water control structures and receives discharge from agricultural fields and 30 wastewater treatment plants. The Central Grand River is particularly influenced by wastewater discharges from five large wastewater treatment plants (WWTPs) in the Region of Waterloo. Due to the impacts of wastewater effluent on the ecological integrity of the Grand River, the Region of Waterloo embarked on a series of upgrades to its two largest WWTPs: the Waterloo and Kitchener plants. This research presents a before-and-after approach used to study and understand the effects of the changes in the operation of the Kitchener wastewater treatment plant. The research documents changes in dissolved inorganic nitrogen dynamics downstream of the Kitchener wastewater treatment plant. Ammonium and nitrate concentrations and nitrogen isotopic composition ($\delta^{15}N$) was monitored in the Central Grand River, with a special focus on the 5700 m reach downstream of the Kitchener wastewater treatment plant effluent over a period of four years (2010 to 2013). The over-riding change in the quality of the wastewater effluent was a decrease in ammonium concentration, resulting in a reduction in the period of oxygen depletion during summer, low flow conditions. After the upgrades, most of the ammonium was oxidized by submerged aeration inside the wastewater treatment plant. However, the concentration of nitrate in the effluent increased as a result of the upgrade to a nitrifying system. The observed rate of ammonium decrease before in the Central Grand River (adjusted by travel time with a flow velocity of 0.3 m/s) varied between 0.7 and 2.47 mgN-$NH_4^+h^{-1}$before the upgrades. Together with the changes in concentrations, the observed differences in the isotopic composition of ammonium ($\delta^{15}N_{NH_4^+}$) and nitrate ($\delta^{15}N_{NO_3}$) suggest that ammonia

volatilization, assimilation and nitrification occurred in the Central Grand River downstream of the Kitchener wastewater treatment plant. Before upgrades, ammonium concentrations in the effluent discharged to the Grand River were higher than 20 mgN-NH_4^+/L and the $\delta^{15}N_{NH_4^+}$ varied between +4 and +10‰. The nitrate concentration in the effluent was frequently between 2 and 4 mgN-NO_3^-/L and the $\delta^{15}N_{NO_3^-}$ from -6 to +1‰. In the 5700 m reach of the river downstream of the Kitchener wastewater treatment plant, the ammonium concentration decreased to between 2 to 0.5 mgN-NH_4^+/L and the $\delta^{15}N_{NH_4^+}$ increased from +5‰ to +30‰. After upgrades (2013), the ammonium concentration in the effluent was low (≤ 6 mgN-NH_4^+/L) due to more efficient ammonium oxidation (and possibly volatilization and assimilation) and the $\delta^{15}N_{NH_4^+}$ was ≈23‰, increasing to +30‰. After upgrades, the nitrate concentration in the effluent was 22 (±5) mgN-NO_3^-/L, and the $\delta^{15}N_{NO_3^-}$ downstream of the Kitchener wastewater treatment plant was between +11 to +14‰. After the upgrades, the nitrate concentration downstream of the outfall from the Kitchener wastewater treatment plant varied likely influenced by intra-annual variations (seasonal variation in temperature) and inter-annual variations (variable river discharge). Downstream of the Kitchener wastewater treatment plant effluent, the dissolved inorganic nitrogen did not return to previously observed background level. The observed differences in the dissolved inorganic nitrogen concentrations among seasons and years were not only attributed to changes in the quality of the WTP's effluent, but also a result of upstream nitrate inputs from agricultural sources.

Ammonium assimilation by epilithon was measured in experimental conditions by blocking bacterial oxidation with a chemical inhibitor (acetylene). Ammonium assimilation was observed at velocities above 1 μm N-NH_4^+ h^{-1}, with a calculated ammonium assimilation rates from 377 to 519 μm N m^{-2} h^{-1}. Nitrate assimilation rates were calculated to be 58 to 65 μmN m^{-2} h^{-1}. Thus, epilithon assimilation contributed from 26% to 100% of the ammonium loss in each experimental unit.

Ammonia volatilization in the Grand River downstream of the effluent before upgrades was estimated to range between 0.61 and 0.13 μgN-NH_3/L per metre, or 0.18 to 0.04 μgN-NH_3/L per second; representing a decrease of approximately 50% of the ammonia discharged from the Kitchener wastewater treatment plant. This is the first time that ammonia volatilization is estimated for a river receiving wastewater treatment plant effluent. Additionally, the ammonia isotopic fractionation factor due to volatilization ($\alpha_{volatilization}$) was calculated experimentally as

1.019 (±0.0024) at pH 8.5, and the kinetic and equilibrium isotopic fractionation factors were calculated as $\alpha_{equlibrium}$=1.036 (±0.0024) and $\alpha_{kinetic}$=1.050 (±0.0024).

A box model that uses ammonium and nitrate concentrations and isotopes of both ammonium and nitrate for estimating the rates of these processes in rivers shows that, before upgrades, the rate constant for gas exchange and ammonium assimilation were similar, however, the change in concentration was larger for ammonium assimilation. After upgrades, the rate constant for nitrification was one order of magnitude higher than before upgrades. The rates estimated by the model for each process , likely changed after upgrades due to the reduced mass of ammonium available for volatilization, oxidation and assimilation. The box model provided constant rates simultaneously estimated for the three processes; thus, the differences between the observed data in this research and the box model are assumed to be the result of: *i)* overestimated volatilization, *ii)* the experimentally measured assimilation on epilithon only and *iii)* the propagation of the error.

Due to the separation between the ammonium ($\delta^{15}N_{NH_4^+}$) and the macrophytes isotopic composition ($\delta^{15}N_{TN}$), it is proposed that some macrophytes and possibly periphyton can be used as an environmental archive that allows one to observe the effects of the wastewater treatment plant effluent discharged into the Grand River. This archive can be used as tool to complement water quality monitoring for assessing changes in water chemistry of rivers and streams receiving wastewater treatment plant effluents.

The most important contribution of this thesis is that it provides a well-documented before-and-after case study of the effects of WWTPs upgrades on the dissolved inorganic nitrogen cycling in an anthropogenically-impacted river. As such, the research provides valuable information that allows regulatory agencies and water managers (i.e., the Region of Waterloo, the Grand River Conservation Authority) to evaluate the impacts and effectiveness of potential upgrades at wastewater treatment plants in order to understand the changes in nitrogen concentrations and loads in receiving waters. This case study can be useful where regional municipalities or regulatory agencies plan to upgrade WTP's in areas with similar geographic and climatic conditions as the observed at in the Central Grand River; however, sampling and monitoring protocols must be designed on a site by site basis taking into consideration baseline conditions and the actual objectives of the final users.

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This is a beautiful river…I often looks into its eyes…I have always learnt from it. One can learn a lot form a river. I have learnt that from the river, everything comes again

Sidhartha (Herman Hesse]

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CHAPTER 1 - GENERAL INTRODUCTION

Nitrogen

Nitrogen is an essential element for all life forms. It is the dominant gas in the atmosphere (78.1% by volume), where it is present in its most stable form, the diatomic molecule N_2 (N≡N). The atmosphere is the largest reservoir of nitrogen (other than the lithosphere), but it is in terrestrial and aquatic ecosystems where the more complex and rapid nitrogen transformations are observed. These include oxidation and reduction sequences driven by microbial activity and environmental conditions (Galloway 2003).

N_2 is not a very chemically reactive form of nitrogen; high energy is required to break its triple bond (226 kcal mol^{-1}). This bond is broken naturally to form more reactive nitrogen compounds by lightning and during bacterial nitrogen fixation (BNF). The high temperatures produced by lightning strikes facilitate the formation of NO from molecular oxygen and nitrogen; which is further oxidized into NO_2 and then into HNO_3. This acidic form of nitrogen is transferred by wet and dry deposition into terrestrial ecosystems within days (Galloway 2003). BNF is completed by a diverse group of prokaryotic bacteria and blue-green algae, expressing nitrogenases (iron-molybdenum enzymes) that reduce N_2 into NH_3 (or NH_4^+), creating *reactive nitrogen* (Swaddle 1990). BNF are free-living cells or cells living in symbiotic association with certain plants (Table 1.1). The Fabacea family contains the most important group of N_2-fixing plants, with around 10000 species, distributed in three subfamilies. Nodules represent infection of the roots by a N_2-fixing bacterium of the genus *Rhizobium*. There are other plant symbioses with woody perennials, cycads, lichens and liverworts (Steward 1977). BNF produces Nr at a higher rate (100 to 290 Tg N yr^{-1}) than lightning (3 to 10 Tg N yr^{-1}, Galloway 2003 and references within).

Bacterial N fixation includes two processes:

$$\tfrac{1}{2}\, N_{2(g)} + 1\tfrac{1}{2}\, H_2O_{(l)} \rightarrow HNO_{3(aq)} \qquad \textit{Reaction 1}$$

$$\tfrac{1}{2}\, N_{2(g)} + 1\tfrac{1}{2}\, H_2O_{(l)} \rightarrow NH_{3(aq)} + \tfrac{3}{4}\, O_{2(g)} \qquad \textit{Reaction 2}$$

Reaction 1 has a small positive free energy of formation (ΔG_f=1780), indicating that, at standard conditions, the reaction will only proceed with the addition of free energy (Burk

1927); thus, the energetically costly reaction 2 is enzimatically completed. When coupling glucose oxidation to nitrogen reduction, these combine to give the following overall equation:

$$C_6H_{12}O_{6(aq)}+4N_{2(g)}+6H_2O_{(liq)}+8H^+_{(aq)} = 6CO_{2(g)}+8NH_4^+{}_{(aq)} \qquad \textit{Reaction 3}$$

With free energy of formation in biological systems ΔG_f= -14 kcal per 0.5 mole N_2 (Bayliss 1956).

Table 1.1 - Nitrogen-fixing organisms and their ecological niches [Adapted from Steward 1977]

Group	Characteristic	Genus
Free-living organism	Heterotrophic bacteria: Aerobes	*Azotobacter, Corynebacterium, Mycobacterium, Pseudomonas, Spirillum*
	Heterotrophic bacteria: Facultative anaerobes	*Bacillus, Klebsiella*
	Heterotrophic bacteria: Anaerobes	*Clostridium, Desulfovibrio*
	Autotrophic bacteria	*Rhodospirillum, Rhodopseudomonas, Chlorobium, Thiobacillus* *
	Blue-green algae: heterocystous	*Anabaena, Calothrix, Cylindrospermum, Nostoc, Stigonema*
	Blue-green algae: non-heterocystous	*Oscillatoria, Plectonema, Trichodesmium*
	Blue-green algae: unicellular	*Gloeocapsa*
Root nodule-forming symbiotes	Leguminous *(Fabacea* spp)	*Rhizobium*
	Non-leguminose angiosperms *(Tremma cannabina)*	*Rhizobium*
	Non-leguminose *(Alnus* sp., *Casuarina* sp., *Myrica* sp.)	Actinomycete
	Cycad - blue-algae	Blue-gren algae
Other eukaryotic organism	Lichens (fungi)	*Nostoc*
	Liverworts *(Anthoceros)*	*Nostoc*
	Pteridophyte (*Azolla*)	*Anabaena*
	Angiosperm	Poaceae - Heterotrophic bacteria

* Chemoautothropic metabolism

There is also an anthropogenic nitrogen fixation process to generate ammonia from nitrogen and hydrogen from gas under controlled conditions using high temperature, high pressure and a

catalyst, through the industrial Haber-Bosch process, which became the dominant source of fertilizers for agricultural purposes by the 1930's. As a result of the increases in population and the high industrial development between 1860 and the recent time, the anthropogenic Nr creation rate increased from 15 TgN per year to 165 TgN per year (Galloway et al., 2002); thus, leading to a global increase in nitrogen in the biosphere.

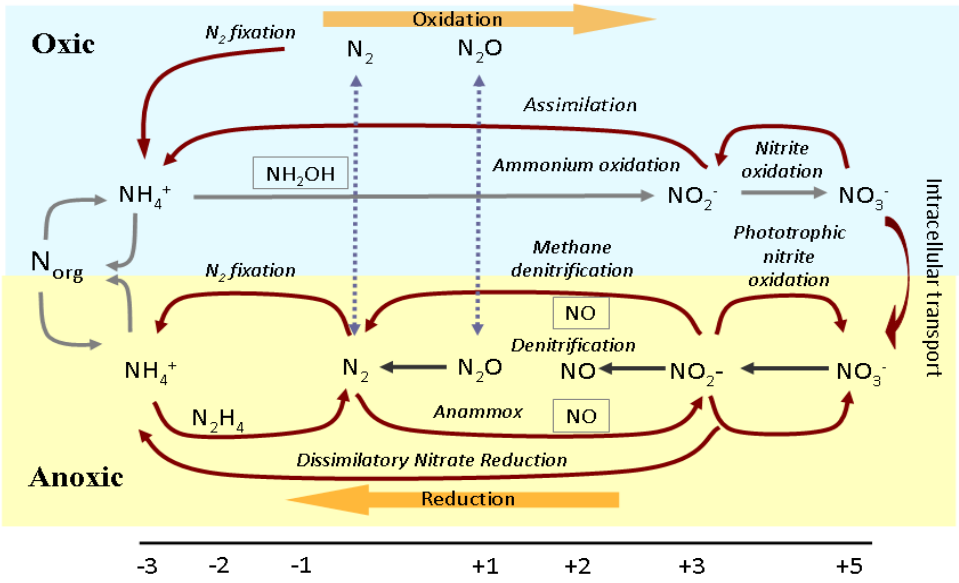


Figure 1.1 - The nitrogen cycle showing processes, N compounds and oxidation states. Adapted from Thamdrup 2012.

Reactive nitrogen (Nr) is a primary nutrient in the ecosystem, for it is required for amino acids, the building blocks of proteins. Nr can be biotically or abiotically oxidized or reduced as its oxidation state ranges from -3 to +5 (Figure 1.1). Despite the fact that nitrogen is an elemental nutrient in the biosphere, the amount of Nr circulating in the biosphere has increased as a result of human activities such as anthropogenic N fixation (Haber Bosch) for agricultural use, increasing of nitrogen-fixing crops, the increase in global population, land use change and burning of fossil fuels, among the most important (Turner et al. 2008 and references within). The increase in Nr has had diverse and complex effects on the soil and water quality in different ecosystems (Turner *et al*. 2008). Consequences of excess Nr include (but not limited to) increased concentration of nitrous oxide (Keuskamp *et al*. 2012), acidification of soil, streams and lakes, proliferation of primary producers and eutrophication (Camargo and Alonso 2006), changes in the taxonomic composition and function oecosystems (Vitousek *et al*. 1997

and references within) and impairment of surface water and groundwater quality (Spalding and Exner 1993).

For around 100 years, food production and combustion of fossil fuels has altered the natural balance of the nitrogen cycle in air, land, and water at all scales. The alteration in the natural balance ranges from extreme nitrogen depletion in some ecosystems to accumulation in other ecosystems (Galloway et al. 2004). The application of synthetic fertilizers (reactive compounds such as nitrates and ammonium salts) or the continuous disposal of treated sewage into aquatic systems, have raised environmental and regulatory concerns as nitrogen accumulates at a rate faster than its reactive species are retired from the ecosystems (Swaddle, 1990). It has been estimated that food and energy production has led to an increase in the anthropogenic Nr creation by factor of 10, compared to the late 19^{th} century (Galloway et al., 2002). At the global scale, terrestrial BNF has decreased 10%, Nr input into rivers has increased 69%, and an overall 77% more nitrogen is exported to coastal areas over the last 70 years (Schlesinger 2009). The single, largest anthropogenic nitrogen input into the biosphere is the Haber-Bosch nitrogen-fixation process, which has created around 1.2 $x10^8$ tonnes per year. Globally, around 10% of the nitrogen applied to the land as fertilizer is incorporated into food, the majority is lost to the environment. Out of this 10%, part of the Nr used in food production is lost after human ingestion via sewage disposal. A large amount of this nitrogen is disposed into freshwater bodies and eventually coastal systems (Schlesinger 2009).

Wastewater treatment plants as nutrients point sources.

Wastewater treatment discharges and septic systems leakages contribute Nr to terrestrial and aquatic ecosystems (Galloway et al. 2004). Human waste makes up a significant fraction of global N exported to coastal zones from rivers (Dumont *et al*. 2005). The Global-NEWS-DIN model indicates that doubling the population density will result in 55% increase in river nitrate exports (Seitzinger *et al*. 2010).

Wastewater treatment plants and septic systems are potential threats to the ecological integrity of ecosystems when they are not properly managed and monitored. Septic tank systems are cost-effective when population density is low, but leakages have increased the amount of organic matter, nutrients, pharmaceuticals and pathogens released into groundwater (Withers et

al. 2014). Wastewater discharges can increase the primary production surface waters, commonly degrading the aquatic ecosystem due to the addition of high organic matter loads, elevating nitrogen and phosphorus concentrations and the simultaneous oxygen consumption (Hagy et al. 2004).

In Canada, the requirements for wastewater effluent discharge are established on a case-by-case basis for each wastewater treatment plant, according to the characteristics of the receiving water body, following both Federal and Provincial regulations. The guideline establishes that secondary treatment or equivalent (*i.e.*, biological conversion of dissolved and organics into biomass and its removal by sedimentation) shall be completed before the effluent is discharged into receiving waters, always considering minimization of adverse health-related and environmental effects, aesthetic nuisance and toxic effects of effluent discharges from heavily populated areas to rivers and streams (Procedure F-5-1, Ontario MOECC, published February 2014). Waiser et al. (2011) mentioned that total dissolved nitrogen (inorganic and organic) concentration above 3mg/L should be described as nitrogen hyper-saturation which could harm or kill certain biota, change the correct ecosystem functioning and render water unfit for drinking purposes at certain times in the year. This condition is found in several rivers, including the Grand River (south western Ontario) with average annual concentrations surpassing this threshold (PWQMN 2011).

The Grand River watershed

The Grand River is a 300 km long river draining into Lake Erie (Laurentian Great Lakes). The Grand River is a seventh order river system located in south-central Ontario, that originates near the Town of Dundalk (525 m.a.s.l.) flows south for 300 km and discharges into Lake Erie, at Port Maitland (173 m.a.s.l.). Its watershed covers an area of approximately 6,800 square kilometres, with an average width of the watershed of 36 km and contributes about ten percent of the drainage to Lake Erie (Figure 1.2). The Grand River is a dendritic river, with several tributaries draining the watershed. The three major tributaries are the Conestogo and Nith rivers on the west, and the Speed River, draining the north-east region of the watershed (GRCA 2013; Lake Erie Source Protection Region 2008. During summer months (mid-June to mid-September), river base-flow is maintained by flow control reservoirs. River flows are monitored by Water Survey of Canada (Environment Canada) and the Grand River

Conservation Authority (GRCA) at diverse locations. Population is around 975 000, half of whom are connected to urban sewage. Due to physiographic differences, the Grand River watershed is commonly divided into three sections: *i)* northern till plains, *ii)* central moraines and *iii)* southern plains with lacustrine influence. The land use is mainly agricultural in the northern area, urban and industrial in the central and agricultural in the south area. The Central Grand River watershed sustains densely populated urban areas: the cities of Guelph, Waterloo, Kitchener, Cambridge and Brantford, together with several townships of the Region of Waterloo.

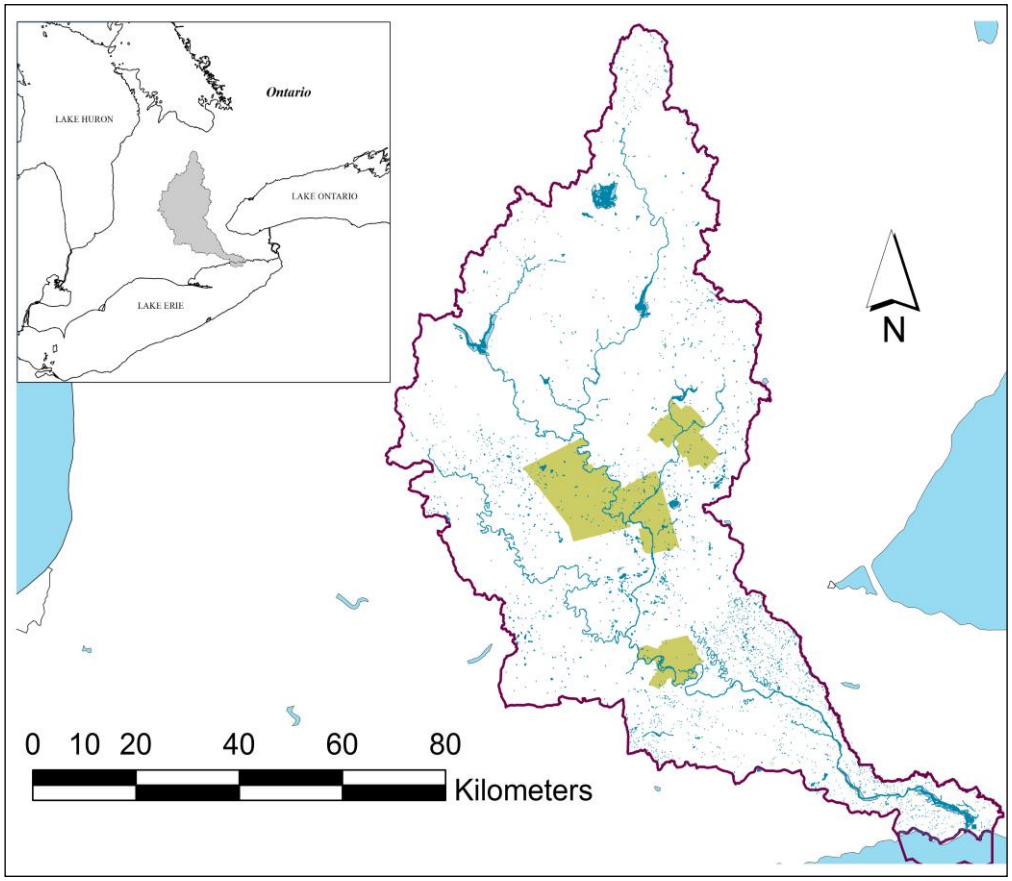


Figure 1.2 - The Grand River watershed, south western Ontario. The Grand River flows 300 km from north (Dundalk) to south (Port Maitland). The colored polygons represent major urban areas.

Geology and dominant soils.

The bedrock of the watershed is mainly Silurian and Devonian limestones and dolostones. These are overlain by unconsolidated glacial sediments throughout most of the basin, dating from the Holocene (up to 11000 years ago). Tills, sand and gravel developed from outwash

and subglacial deposits in the north part of the watershed, whereas glaciolacustrine clays and silts are found in the south (Easton 1992; Nelson et al., 2003).

Vegetation and land use

Before the watershed was cleared for agriculture, it was almost entirely forested, but is now between 12 to 25% covered in forest. Since European colonization, about 70% of the original marshes and wetlands have been drained, decreasing water storage capacity, promoting rapid runoff and uneven stream flow (Nelson et al. 2003; Lake Erie Source Protection Region, 2008). There are no large areas untouched by human activities. The land use is mainly agricultural in the north and south areas, urban and industrial in the central area. The agricultural land is used to grow cash crops (corn, soy and cereals), hay, fruits, vegetables, and sod or nursery crops (Region of Waterloo Census Bulletin 2011). The relatively young soils of Ontario are thin and sensitive to erosion if disturbed and not properly managed (Easton 1992). Within the watershed, the Grand River conservation Authority (GRCA) has been responsible for reforestation, water management and creation of conservation areas (Nelson et al. 2003) which has been successful in maintaining and improving the quality of the river and the adjacent land.

Climate

The Grand River is divided into four climate regions. These are (from north to south) the Dundalk Uplands, the Huron Slopes, the South Slopes and the Lake Erie Counties (Lake Erie Source Protection Region, 2008). Colder and longer winters are observed in the hedwaters and moist and warmer climates close to the mouth. A large amount of the precipitation falls in form of snow in winter. This region is affected by lake effects from the Great Lakes, jet streams, high and low pressure cells and weather coming from the Arctic and the Gulf of Mexico (Lake Erie Source Protection Region, 2008). According to Weather Canada (Region of Waterloo International Airport-6149388) the daily temperature (2002-2006) oscillated from -10.4 to 21.6 ºC. The annual average precipitation is around 750 mm (506 -952 mm).

Urban impacts

The Grand River watershed has a long history of settlement that has drastically altered the landscape and impacted surface water and groundwater quality and quantity. Some channels

are now impervious and the watershed has about 34 water control structures, from overflow weirs to large dams and reservoirs (Lake Erie Source Protection Region, 2008). Approximately 93% of the land draining into the Grand River is rural, yet 81% of the total population lives in the urban area (GRCA, 2013) which represents important point sources of pollution. Those impacts are measurable in terms of households connected to the sewage system, volume of treated water discharged into the Grand River or its tributaries and treatment costs. Most municipalities in the watershed use groundwater for the domestic water supply; for example, 80% of the water used by the city of Cambridge is groundwater, whereas the remaining 20% comes from the Grand River (http://www.cambridge.ca/planning_and_development_department/policy_planning/cambridge_waters). On the other hand, the city of Brantford draws all its water supply from the Grand River (Lake Erie Source Protection Committee 2015), which requires that the intake water has some minimum quality standards in order to be treated and distributed for human consumption.

The watershed population is expected to increase to around 1.1 million inhabitants by the year 2031 (GRCA 2013); which represents an increased in the volume treated by any of the 30 wastewater treatment plant in the watershed. The Grand River receives agricultural and urban pollution from both point (sewages plants, field tiles) and non-point sources (agricultural runoff fertilizers). Historically, the Grand River has experienced low oxygen concentrations during summer, due to the high organic load (MacCrimmon and Kelso 1970, Rosamond 2013). The Region of Waterloo upgraded two of its largest wastewater treatment plants (Waterloo and Kitchener), which represented changes in quality of their effluent, namely reduced ammonia concentration with the consequent improvement in the dissolved oxygen in the river.

Of particular importance are the upgrades proposed and completed for the Kitchener wastewater treatment plant due to the proposed changes to the quality of the effluent. The Kitchener wastewater treatment plant (KTP) is a conventional secondary wastewater treatment plant that performed an activated sludge process with chemical phosphorus removal, anaerobic sludge digestion and sodium hypochlorite disinfection. The KTP discharged effluent with high ammonium concentration, causing oxygen depletion in the Grand River during summer, low flow conditions (Figure 1.3); thus, did not fulfil the objective limits for water quality in the

Grand River (Table 1.2). By 2013, after the majority of the upgrades have been completed, full nitrification was expected and a completely new secondary treatment plant is projected for 2018 (Region of Waterloo 2012).

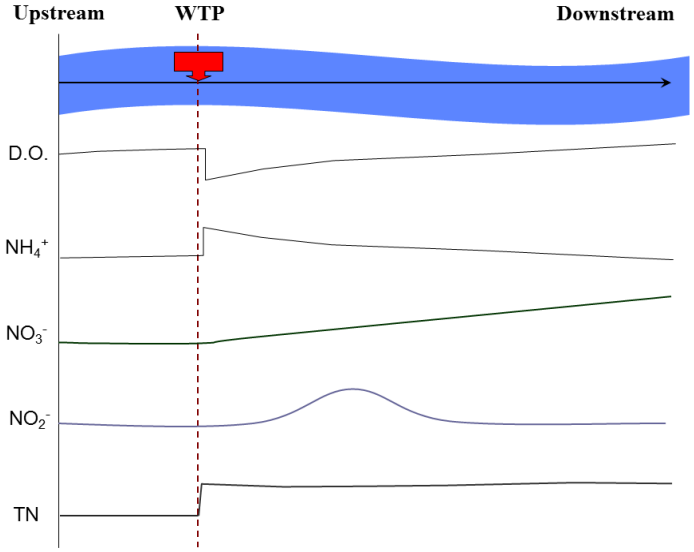


Figure 1.3 - Conceptual nitrogen cycle in the Central Grand River downstream of a wastewater treatment plant (WTP) before upgrades. The lines represent dimensionless longitudinal behaviour; no seasonal trends have been included.

Table 1.2 - Objective limits for water quality parameters in the Grand River following regulatory bodies guidelines. Modified from Loomer and Cooke 2011.

Parameter	Objective (mg/L)	Jurisdiction
Dissolved Oxygen (25ºC)	4	Ontario Ministry of the Environment
Nitrates (NO_2^-+NO_3^-)	2.93	Canadian Environmental Quality Guideline
Nitrate (NO_2^-)	0.06	Ontario Ministry of the Environment
Ammonia (NH_3)	0.019	Ontario Ministry of the Environment
Total Phosphorous	0.03	Ontario Ministry of the Environment

Stable isotopes as environmental tracers

Isotopes are atoms of the same element that have different numbers of neutrons, and the difference in the number of neutrons conveys differences in their mass (Kendall and Caldwell 1998). Stable isotopes do not show large differences in the number neutrons and comprise the bulk of the atoms of a given element in earth and moves into different reservoirs; thus, they are

continuously incorporated and recycled among different compartments, either organic or inorganic. Nitrogen has two stable isotopes: ^{14}N and ^{15}N, this last comprises 0.3663% of the total N abundance. Given the small amount of ^{15}N in the biosphere, the stable isotope ratio *R* is used for expressing the number of the heavy isotope (^{15}N) respect to the number of the light isotope (^{14}N) in a substance *A*; $R=\left({}^{15}N/{}^{14}N\right)_A$. Consequently, the relative differences of isotopes ratios R between two substances is called the isotope delta, which is the relative differences of isotope ratios expressed as

$$\delta^{15}N_{P/Q}=\left(\frac{\left({}^{15}N/{}^{14}N\right)_P-\left({}^{15}N/{}^{14}N\right)_Q}{\left({}^{15}N/{}^{14}N\right)_Q}\right)$$

Where the substance *P* is an environmental sample and *Q* is the reference material, which is atmospheric nitrogen for this particular example. The isotope delta is also known as isotope delta and is reported in parts per mil (‰; Robinson 2001, Coplen 2011).

Given the large range of oxidation states in nitrogenous species (from -3 to +5), the nitrogen isotope delta ($\delta^{15}N$) in solid, liquid and gaseous phases varies due to biological, physical and chemical reactions. These reactions can be reversible or irreversible and impart an apparent distribution of isotopes between the phases, called the isotopic fractionation ε. The isotopic fractionation is a quantity commonly used to describe the distribution of isotopes between molecules of a compound, among phases or among reactants and products (Coplen 2011). The isotopic fractionation ε is derived from the isotopic fractionation factor α, the ratio of ratios of the heavy isotope relative to the light isotope, commonly expressed as $\alpha_{P/Q}=\frac{R_P}{R_Q}$.

The processes that change the nitrogen isotopic composition include ammonia volatilization, assimilation, nitrification and denitrification. All these processes yield different relative isotope deltas; thus, producing ^{15}N-enriched or ^{15}N-depleted forms of nitrogen in solution, in gases and in the submerged biomass due to preferential mobilization of the light isotope relative to the heavy isotope (*i.e* discrimination against ^{15}N). The isotope deltas of ammonia (NH_{3g}), ammonium (NH_4^+), nitrate (NO_3^-) and biomass (TN) were used for gaining a better

understanding of the physical, chemical and biological processes occurring in the central Grand River before, during and after the upgrades in the KTP at different spatial scales and seasons.

Thesis outline

The Grand River receives urban inputs from both point and non-point sources. Wastewater treatment plants as nutrients point sources are of particular concern in the Central Grand River. Improving the quality of the effluent and decreasing the impacts of the effluent in the ecosystem health of the Grand River were the objective of the KTP upgrades (Region of Waterloo 2013). These upgrades were achieved in multiple phases over several years, which facilitated the study of large part of the transition for a period of four years.

Previous studies have addressed water quality concerns in the Grand River, with particular emphasis on nutrients (Barlow-Busch *et al*., 2006, Loomer 2009, Hutchins 2011, Rosamond *et al*., 2011, Hood et al. 2014, Morrison 2014, Shaker 2014) and oxygen dynamics (Jamieson 2010, Chen 2013). This thesis focuses on the effects of wastewater treatment plant effluent upon the dissolved inorganic nitrogen dynamics in the Central Grand River before, during and after upgrades in the KTP.

The objective of this thesis is to describe the changes in the dissolved inorganic nitrogen dynamics in the Grand River downstream of the KTP (the largest treatment plant in the Region of Waterloo) as a result of the upgrades to both the Kitchener and Waterloo wastewater treatment plants, including different the spatial and temporal components. The upgrades in the WTP's of the Region of Waterloo makes the Grand River a good case study to describe and understand the changes in the aquatic ecosystems in response to wastewater effluent with different water quality.

This research comprise four years of field sampling and data collection, which includes before (2010-2011), during (2012) and after (2013) upgrades in the KTP studying the effects in small scale (5.7 km downstream of the KTP effluent) and at the Central Grand River, and covers seasonal patterns of dissolved inorganic nitrogen and the isotopic composition of ammonium, nitrate and biological organisms. Nitrogen stable isotopes of ammonium and nitrate were used to investigate the physical, chemical and biological processes occurring in the river. Laboratory

experiments provided evidence about the role of biological assimilation and isotopic fractionation during ammonia volatilization. Finally, estimates of different processes are provided for the Central Grand River as an example of the outcomes after completing upgrades on a wastewater treatment plant discharging into a river within an urban area.

The thesis includes an introductory chapter (*chapter 1*), five research chapters (c*hapter 2 through 6*) and an integrative chapter (*chapter 7*). The specific objectives of each chapter are discussed below.

Chapter 2 presents an experiment designed to assess the ammonium and nitrate assimilation by epilithon. Ceramic tiles were colonized with the autochthonous epilithic community of the Central Grand River during summer, taken to controlled conditions for measuring ammonium and nitrate uptake over a short period (48 hours).

Chapter 3 includes a series of experiments at two pH values, in order to assess the isotopic fractionation associated to ammonia volatilization in controlled conditions.

Chapter 4 includes the characterization and description of the plume of nutrients produced by the KTP effluent before and after upgrades, particularly during summer, low flow conditions, when the plume is least diluted and the environmental impacts are greater. It was estimated the length of the plume of nutrients and includes the trends of ammonium and nitrate concentrations in the Grand River downstream of the KTP effluent during the day and night. Simultaneously, the observed changes in ammonium and nitrate isotope delta contributed to the understanding of the processes driving the nitrogen cycle downstream of the KTP before and after upgrades. An estimate of the contribution of ammonia volatilization to the overall ammonium decrease in the Grand River is provided for the first time.

In Chapter 5, it is shown that macrophytes and periphyton can be used as an environmental archive given that the changes in the nitrogen isotopic composition in the wastewater treatment plant effluent where also observed as changes in the isotopic composition of the biological organism. Part of this chapter has been published as “Epilithon isotope composition as an

environmental archive in rivers receiving wastewater: the case of the Grand River, Ontario, Canada" in the *Journal of Water Science* (Volume 27-3; eISSN: 1718-8598).

Chapter 6 assesses the effects on intra- and inter-annual variations in the dissolved inorganic nitrogen observed in the Central Grand River before and after upgrades in the operation of two of the largest wastewater treatment plants in the Region of Waterloo.

Finally, chapter 7 combines data from all previous chapters, providing an estimate of the potential contribution of ammonium oxidation, ammonia volatilization and nitrate and ammonium uptake to the dissolved inorganic nitrogen dynamics in the Central Grand River. The results reported in this thesis aim to assist administrative agencies, Conservation authorities and decision-makers on the outcomes observed after upgrades of a wastewater treatment plant discharging into a river within a heavily populated area.

CHAPTER 2 -THE ROLE OF PERIPHYTON ON THE NITROGEN CYCLING DOWNSTREAM OF A WASTEWATER TREATMENT PLANT

INTRODUCTION

Periphyton is the taxonomically diverse assemblage of aquatic organisms (bacteria, fungi, protozoa, algae and invertebrates), detritus and sediments attached to a solid substrate. Periphyton includes plankton that becomes sessile or attached onto biotic and abiotic surfaces (Azim et al. 2005). Several names have been assigned to this community according to the substrate on which it growths; epiphyton if grows onto aquatic plants, epipelon when it grows on top of sediments, epixylon if develops over wood, episammon when the substrate is sand and epilithon when it grows on the surface of rocks. Substrate in the central Grand River is predominantly cobbles; therefore, the role of the epilithic community in nitrogen cycling is of primary interest in the present research.

The aspect of the epilithic community varies from crust to mats and filaments in which the colour suggests the possible taxonomical composition. Diatoms can be the most important algal component of periphyton (90% of total biovolume, Azim and Asaeda 2005), but also other algae taxa and bacteria can be dominant in epilithon (Araya et al. 2003).

Due to its complex taxonomic composition, the epilithon is a significant primary producer and nutrient cycler in aquatic systems (Wetzel 1964). Its relative contribution to the overall productivity depends on several factors such as light intensity, grazing pressure, nutrient availability, alkalinity, the hydraulic properties of the channel and the land use of the catchment area (Axler and Reuter 1996, Azim and Asaeda, 2005). Epilithon can generate biologically available nitrogen by N-fixation, assimilate reactive nitrogen from the water column or nitrify ammonium. Nitrogen fixing organisms include several bacteria families that generate energy from a number of metabolic processes (Gordon 1981). The most common prokaryotes fixing nitrogen are free-living Cyanobacteria of the genera *Anabaena*, *Calothrix, Amphithrix, Dichothrix, Schizothrix, Rivularia* and *Nostoc* (Vitousek et al. 2002) and endosymbiotic Cyanobacteria living in association with some diatoms (Fairchild et al. 1985). Reactive nitrogen (Nr) at different redox state can be recycled by nitrification (ammonia oxidation),

denitrification, ammonium and nitrate assimilation into biomass (*i.e*. uptake) and mineralization of organic matter that regenerates ammonium (Galloway 2003).

The epilithic colonization process is gradual, sharing some common features with other substrates. First, a coating of dissolved organic substances (such as amino acids and mucopolysaccharides) forms on the substrate; then, a coating of bacteria begins to form (Azim and Asaeda 2005). Dissolved and suspended particulate organic matter is a nutrient source for bacteria, which actively attach and secrete mucilaginous strands that create more binding sites for more organic and inorganic material (Azim and Asaeda 2005). Bacteria are typically observed to colonize first; diatoms are commonly next, particularly stalked diatoms (Azim and Asaeda 2005). A climax community includes filamentous algae forming a layered community where grazers, predators and scavengers are found (Azim and Asaeda 2005; Wetzel 2005). The complexity of the epilithic community increases gradually over time, controlled by grazing, detachment and sediment blasting (Azim and Asaeda 2005).

Several nitrogen transformations can occur in the multilayered epilithic community; thus chemical inhibition can be used to block certain bacterial processes to investigate processes that are not affected by chemical inhibition. For instance, several substances inhibit nitrification, either because of a biocidal activity or by causing toxicity to specific microbes that oxidize ammonium (Huber et al. 1977). The activity of the enzyme ammonia monooxygenase (AMO, which oxidizes ammonium into hydroxylamine and finally into nitrite), can be reduced or blocked by direct binding with alternative substrates, such as acetylene. Direct binding with alternative substrate results in irreversible inactivation of the enzyme and recovery involves *de novo* protein synthesis (McCarty 1999). This type of chemical inhibition was used in this research to distinguish plant uptake from ammonia oxidation in the epilithic community.

Nitrogen transformations mediated by periphyton have been studied in engineered ecosystems and wastewater facilities (Jeanfils et al. 1993; Ogura et al. 2009). Understanding the role of epilithon in the N cycle downstream of the discharge of wastewater into the Central Grand River is central for this thesis, given that two of the largest wastewater treatment plants in the Region of Waterloo (Waterloo and Kitchener WTP's) have been upgraded to ensure better

effluent quality (Region of Waterloo 2012). Treatment upgrades are measurable since early 2013, succeeding in reducing ammonium and alleviating dissolved oxygen depletion downstream the urban area of the Region of Waterloo (Region of Waterloo 2013b). As a result of those upgrades, nitrate concentrations increased relative to background levels; therefore, the central Grand River downstream of the urban area has lower-than-before ammonium and higher-than-before nitrate concentrations.

The objective of this chapter was to experimentally measure the epilithon contribution to ammonium and nitrate transformations in the central Grand River. It was hypothesized that ammonium uptake by epilithon was an important component of the total NH_4^+ decrease observed in the central Grand River before upgrades, and that the ammonium uptake was higher than nitrate uptake. According to the conceptual model depicted in Figure 2.1, if a closed system contains equal amounts of ammonium and nitrate, any decrease in the total inorganic nitrogen (TIN=$NO_2^- + NO_3^- + NH_4^+$) represents net ammonium and/or nitrate uptake. If the TIN did not change, then only nitrification was occurring as the ammonium is oxidized into nitrate. Laboratory experiments were conducted to measure short-term (48 h) ammonium and nitrate uptake by epilithon in small chambers. The net contribution of ammonium uptake was quantified by blocking bacterial ammonium oxidation with acetylene. When nitrification was blocked, nitrate is not produced and the decrease in ammonium is solely attributed to uptake.

MATERIAL AND METHODS

In situ biomass estimation

In situ estimation of epilithic biomass was completed at four locations in the Central Grand River: one location upstream of the Kitchener wastewater treatment plant (Victoria St. 14 km upstream of the Kitchener effluent) and three locations downstream of the effluent. Two sampling locations were close to the KTP effluent (520 and 880 m) and the third one (5700 m) is a location established as monitoring station for the Grand River Conservation Authority (GRCA) and the Provincial Water Quality Monitoring Network (PWQMN). In each location, randomly selected $1m^2$ plots were delineated with a metallic frame to estimate the colonized riverbed area (five plots per location). Each plot was subdivided into four quadrats and the

epilithic biomass from all the cobbles in one quadrat ($0.25m^2$) was collected for biomass estimation by scraping and brushing the surfaces of the rocks. The material scraped from the rocks was a green slurry containing filamentous algae and mineral particles. Ash-free dry weight (AFDW) was obtained by drying the biomass for 24 h at 60ºC, weighing it, baking it for 2 h at 550ºC and then reweighing. Biomass per square meter was estimated by extrapolating the biomass measured in 0.25 m^2 to the total surface covered with cobbles in each $1m^2$ plot.

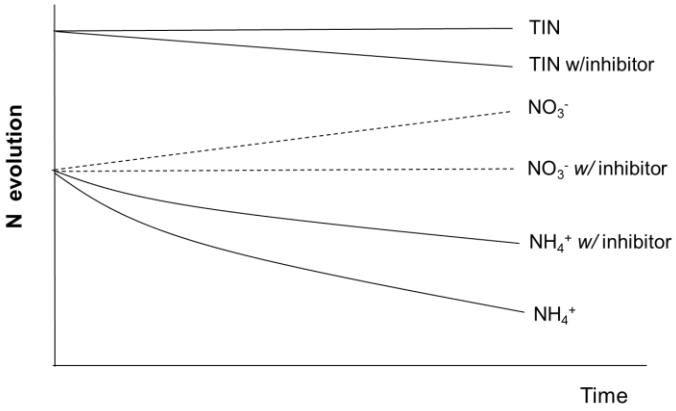


Figure 2.1 - Conceptual model of expected trends in ammonium and nitrate by epilithon in small chambers that initially contain equal amounts of ammonium and nitrate. The inhibitor used was 1% acetylene (v/v).

Growth of biomass on tiles

Epilithon was grown on unglazed ceramic tiles of 4 in^2 (25.8 cm^2). Several sheets of tiles were incubated in uncovered plastic containers in the Grand River at a location 520 m downstream of the Kitchener wastewater treatment plant (KTP henceforth) during June and July 2013. Tiles were located near the south bank of the river, outside of the KTP effluent plume. Flow velocity and water depth varied considerably due to the large amount of rainfall during the incubation period. Biomass was grown for 35 day, similar to a study on epilithic phosphate uptake in the upper Grand River (Barlow-Busch *et al*. 2006). The material was scraped from the tiles with a soft brush and a spatula. The green slurry obtained included biomass and mineral particles; no obvious filamentous algae were observed. The algal and bacterial communities were not evaluated, however it has been previously reported for the Grand River that similar tiles showed initial colonization by *Cladophora*, diatoms and an unidentified green alga (Barlow-Busch et al. 2006).

AFDW was obtained by scraping the surface of 16 tiles with soft brush and spatula, drying the material for 24 h at 60ºC, weight and then furnace-baking for 2 hours at 550ºC and reweighing the material. No carbonate removal step was performed. AFDW together with chlorophyll *a* (see below) were used to estimate initial epilithon biomass.

Chlorophyll estimation

Chlorophyll from tile slurries was measured with the trichromatic method. Biomass was scraped and brushed from four tiles to obtain slurry that was taken to 500 mL of water and kept cold and dark until analysis. The samples were filtered (0.5-0.7 bar) onto a pre-baked Whatman GF/F filter (0.7 μm). After filtration, filters were folded in half and placed in a 20 ml glass vial. Pigments were extracted with 10 mL of 90% acetone, gently shaken and stored in darkness overnight (4ºC). Before analysis, samples were filtered to remove glass fibres in the liquid. The absorbance of extracts was measured at 750, 664, 647, and 630 nm; acetone blanks were also measured. Chlorophyll concentrations were calculated using the following equations (Jeffrey and Humphrey 1975).

$$chl\,a = \frac{[11.85 \times (E_{664} - E_{750}) - 1.54 \times (E_{647} - E_{750}) - 0.08 \times (E_{630} - E_{750})] \bullet V_e}{L \times V_f}$$

$$chl\,b = \frac{[-5.43 \times (E_{664} - E_{750}) + 21.03 \times (E_{647} - E_{750}) - 2.66 \times (E_{630} - E_{750})] \bullet V_e}{L \times V_f}$$

$$chl\,c = \frac{[-1.67 \times (E_{664} - E_{750}) - 7.6 \times (E_{647} - E_{750}) + 24.52 \times (E_{630} - E_{750})] \bullet V_e}{L \times V_f}$$

Where V_e is the volume of acetone used (mL), V_f is the volume of water filtered (L) and L is the path length of the cuvette (1 cm, Beckman 530 spectrophotometer). Chlorophyll was expressed in mg chl m^{-2}. The surface area of the unglazed ceramic tiles was 25.8 cm^2).

Description of the experiment

Ceramic tiles were placed in the Grand River for 35 d to allow biomass accrual and growth. The colonized tiles were then brought back to the laboratory at the same time for the experiments (Table 2.1). The tiles used for the "*Uptake*" treatments were pre-incubated in a solution of 1% acetylene (v/v) overnight to inhibit of nitrifying bacteria (Herrmann *et al*. 2007).

All treatments began with 300 mL of nitrogen-enriched (ammonium, nitrate and ammonium+nitrate) river water filtered to 0.45 μm in 400 mL HDPE containers (Starplex®). The nitrogen concentrations were similar to concentrations measured in the Grand River at locations upstream and downstream relative to the KTP effluent, before and after upgrades, except for high ammonium, which was higher than had been observed in the Grand River from 2010 to 2013.

Table 2.1 - Experimental design and initial conditions for assessing ammonium and nitrate uptake in epilithon. Nitrogen species refers to the dominant for of nitrogen by treatment. Acetylene (1% v/v) was added to some treatments to inhibit nitrification. *n.d.* - under detection limit (0.05 mg N/L)

Treatment	N sp.	Level	Condition	$N\text{-}NH_4^+$		$N\text{-}NO_2^-$		$N\text{-}NO_3^-$	
				μmol	mg/L	μmol	mg/L	μmol	mg/L
EC-A	NH_4^+	Low	Acetylene	79	1.1	1	0.02	93	1.3
EC-B	NH_4^+	Low		79	1.1	1	0.02	93	1.3
EC-C	NH_4^+	Mid	Acetylene	221	3.1	1	0.01	93	1.3
EC-D	NH_4^+	Mid		221	3.1	1	0.01	93	1.3
EC-E	NH_4^+	High	Acetylene	336	4.7	*n.d.*	*n.d.*	93	1.3
EC-F	NH_4^+	High		336	4.7	*n.d.*	*n.d.*	93	1.3
EC-G	NO_3^-	Low		*n.d.*	*n.d.*	114	0.16	79	1.1
EC-H	NO_3^-	Mid		*n.d.*	*n.d.*	107	0.15	243	3.4
EC-I	NO_3^-	High		*n.d.*	*n.d.*	*n.d.*	*n.d.*	123	5.7
EC-J	NH_4^++NO_3^-	Low	Acetylene	79	1.1	1	0.01	79	1.1
EC-K	NH_4^++NO_3^-	Low		79	1.1	1	0.01	79	1.1
EC-L	NH_4^++NO_3^-	Mid	Acetylene	170	2.5	1	0.01	229	3.2
EC-M	NH_4^++NO_3^-	Mid		170	2.5	1	0.01	229	3.2
EC-N	NH_4^++NO_3^-	High	Acetylene	279	3.9	1	0.02	350	4.9
EC-O	NH_4^++NO_3^-	High		279	3.9	1	0.02	350	4.9

All treatments started at the same time with the following conditions: 25º C, 75% humidity, 24 h light regime (630 lumens, incandescent bulbs), continuous stirring. Containers were closed but not sealed, and each tile was elevated 2 cm from the bottom with a plastic lid (Figure 2.2). 5 ml sub-samples were collected at 0, 0.5, 1, 2, 3, 6, 12, 24 and 48 h. The results were modeled to curves with a zero-order rate ($N= N_0 + k \cdot t$) that represented the uptake rate *V* (velocity) in μm N h^{-1}. Changes in biomass during the experiments (48 h) were not evaluated in any treatment.

Figure 2.2 - Experiment set-up in laboratory conditions (left) and one experimental unit with tile and treatment code (right). All treatments began with 300 mL of nitrogen-enriched river water, filtered to 0.45 µm in a 400 mL Starplex® HDPE containers. Ambient conditions were 25º C, 75% humidity, 24 h light regime (630 lumens, incandescent bulbs), continuous stirring.

A parallel experiment assessed the effect of light on nitrate and ammonium uptake, using a full factorial experiment (2^3) to evaluate the intensity of nitrate and ammonium uptake at two concentration levels (low 1.09 mgN-NH_4^+/L and 1.07 mgN-NO_3^-/L; high 4.71 mgN-NH_4^+/L and 5.75 mgN-NO_3^-/L) and illumination conditions (light and darkness; 24 h light regime, 630 lumens, incandescent bulbs). Changes in biomass were not evaluated.

In order to support ammonium preference over nitrate, the rate of preferential incorporation (RPI) was used (Dortch 1990)

$$RPI_{NO_3^-} = \frac{P_{NO_3^-} / \Sigma\rho_N}{[NO_3^-] / [\Sigma N]}$$

Where P_{NO3}^- is nitrate uptake rate, $\Sigma\rho_N$ is the sum of rates of all nitrogen species, [NO_3^-] nitrate concentration and [ΣN] is the sum of all nitrogen concentrations. Values less than 1 indicate preference for ammonium. The equation is equally valid when total ammonium nitrogen TAN is used instead of NO_3^- (Takahashi and Saijo 1981).

Statistical analyses

All statistical analyses were performed in JMP 5.1 (SAS Institute). For the custom factorial design, repeated measures ANOVA was performed in order to assess the effect of

concentration level, nitrogen species and inhibitor over the response variables ammonium and nitrate over time. Effect tests (F) and Least Squares Mean plots were produced to assess significant effects of the variables and their interactions. The effect of light was analysed as a full factorial design (2^3) to assess the effect of the light, concentration and interactions over ammonium and nitrate assimilation.

RESULTS

Epilithon biomass estimates

In situ biomass estimation

The location upstream of the KTP effluent had large variability in biomass and was the location with the highest average biomass (51.6 g biomass DW m^{-2}) compared to the locations downstream of the KTP (8.8 to 29.2 g biomass DW m^{-2}, Figure 2.3). Pooling all the samples collected downstream of the KTP effluent, the average epilithic biomass in the summer of 2012 was 19.3 g DW m^{-2} (median=13.7 g DW m^{-2}). Chlorophyll was not estimated for cobbles; however, the *chla:biomass* ratio measured on the tiles was 0.06%. The biomass per unit surface in the field was lower than on the tiles that were incubated for 35 days.

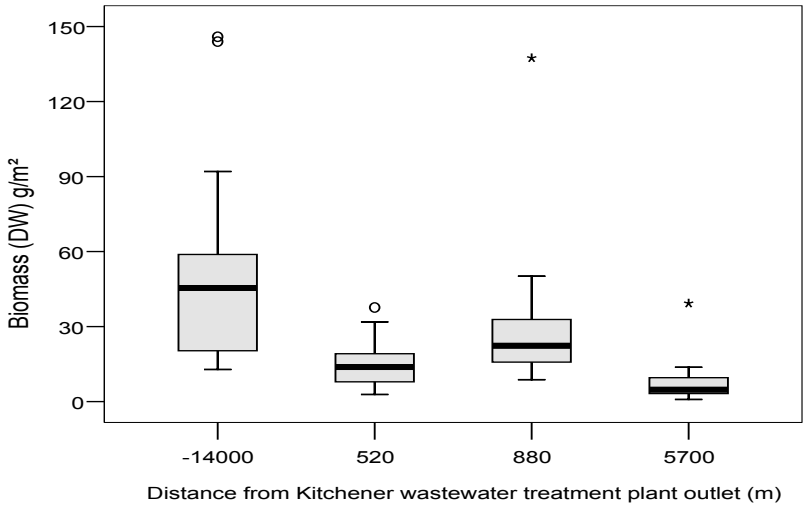


Figure 2.3 - Epilithon biomass estimated in the field in August 2012 from 0.25 m^2 plots in the Central Grand River at different location relative to the Kitchener wastewater treatment plant effluent. Boxplots show the median (*black line*), interquartile range (*boxes*), points outside the interquartile range (*circles* and *asterisks*).

Epilithic biomass growth on tiles

Biomass (AFDW) on tiles was between 16 and 123 g dry weight m^{-2} (73.6 ± 37.2 g m^{-2}). Estimates of chlorophyll for the tiles were: 42.4 ± 9.6 mg *chl a* m^{-2}, 8.7 ± 3.9 mg *chl b* m^{-2} and 3.6 ± 1.7 mg *chl c* m^{-2}. The *chla:biomass* ratio was 0.06%.

Ammonium uptake

The change in ammonium concentration ($NH_4^+{}_{initial} - NH_4^+{}_{final}$) was significantly different among treatments (Tukey Kramer HSD, p=0.01, df=12). *Uptake only* treatments showed a less steep decrease in ammonium (Figure 2.4, upper panel) and only in the experimental units at the lower ammonium concentrations (1.1 mg N-NH_4^+/L) ammonium was assimilated completely in the 48 h duration of the experiment. In the *Uptake+Oxidation* treatments, almost all of the ammonium was consumed; only the highest concentration treatment (> 4mg TAN/L) had ammonium above the detection limit (0.05 mgN-NH_4^+/L) after 48 h (Figure 2.4, lower panel).

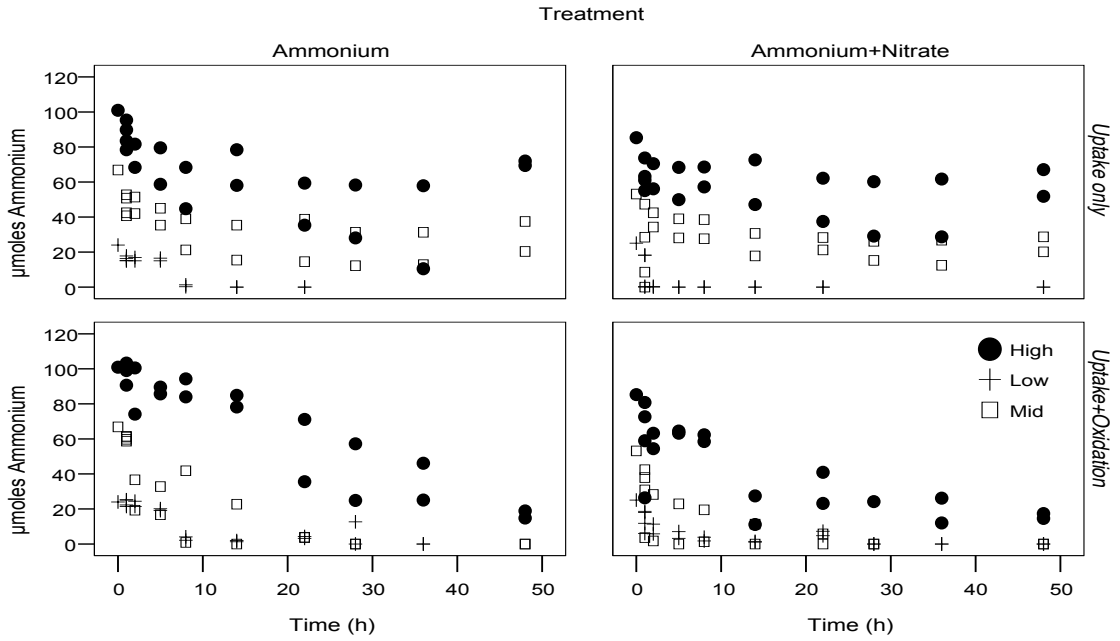


Figure 2.4 – Decrease in ammonium concentration (µmoles N-NH_4^+) with time at different concentration levels. Acetylene inhibition is represented in *Uptake* only treatment (lower panels). Concentration levels as described in Table 2.1.

Thus, ammonium uptake represented 46% and 56% decrease of the initial ammonium concentration in the mid and high concentration treatments respectively. The decrease in ammonium by treatment (in µmoles N-NH_4^+ h^{-1}) showed differences between assimilation

(*Uptake only*) and the combination of assimilation and nitrification (*Uptake+Oxidation*). It is important to highlight that some experimental units of the same treatment showed different assimilation patterns (compare circles at mid and high concentration levels, Figure 2.4).

Nitrate uptake

Treatments with nitrate as the only source of nitrogen showed moderate NO_3^- decrease. There was an unexplained variation in NO_3^- concentration in the high-NO_3^- treatment at eight h into the experiment, but it was almost stable at low and mid concentrations (Figure 2.5). These trends suggested that nitrate was consumed in the high and mid concentration treatment and produced in the low concentration treatment, despite the fact that nitrate was the only source of nitrogen available in the experimental unit. When nitrate declined during the experimental, the loss was between 5 and 11% of the initial nitrate in the high and mid concentration treatments, respectively.

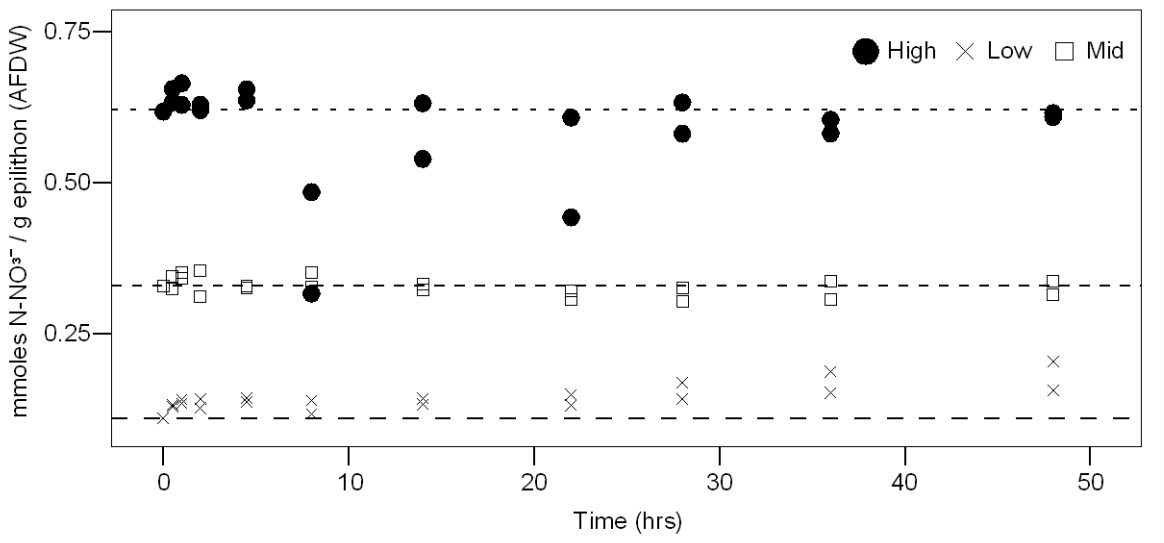


Figure 2.5 - Biomass-weighted nitrate decrease at three different nitrate concentrations with nitrate as only nitrogen source. Deviations from the lines ($NO_{3\ initial}^-$) represent nitrate consumption (below the line) or production (above the line). Nitrogen mass by treatment is described in Table 2.1

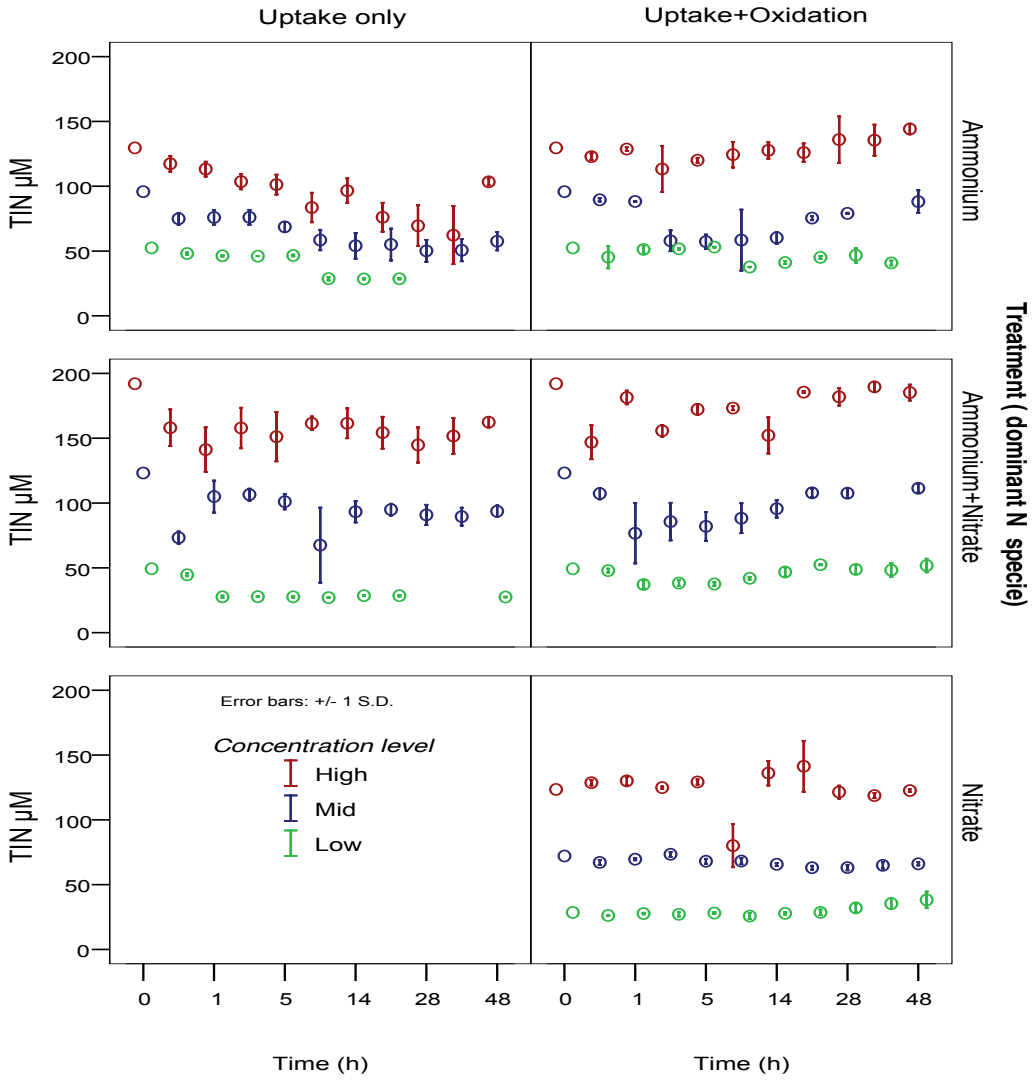


Figure 2.6 - Total inorganic nitrogen (NO_2^-+NO_3^-+NH_4^+) with time in the various treatments. The right axis indicates the nitrogen treatment: ammonium (upper panel), ammonium+nitrate (central panel) and nitrate (lower panel). Treatments with acetylene inhibition are represented in *Uptake only* graphs (*left column*). Nitrogen mass addition by treatment is described in Table 2.1. Error bars represent ± 1 standard deviation of two replicates.

Changes in the total inorganic nitrogen

The decrease in TIN was significantly different with time between the *Uptake* and *Uptake+Oxidation* treatment (ANOVA $F = 24.2$, $p < 0.0001$, $df=26$). The decrease in the total inorganic nitrogen (TIN) in the treatments inhibited with acetylene provided evidence that the acetylene inhibition resulted in ammonium uptake (Figure 2.6, upper panel). The treatments with NO_3^-+NH_4^+ showed variable TIN concentrations throughout the experiment with a slight

decrease by the end of the experiment (Figure 2.6, mid panel). The TIN was relatively constant in the nitrate treatment (Figure 2.6, lower panel). The time and the concentration level were also different among treatments, but these results are trivial since changes in mass over time were also expected as a result of ammonium oxidation and uptake.

Canonical denitrification (*i.e*. the reduction of N-oxides coupled to electron transport and phosphorylation) was assumed to be negligible, based on the dissolved oxygen measured (6.9 mg O_2/L, 78% saturation). Nitrogen fixation and ammonia volatilization were not considered in these calculations.

The interaction of NO_3^- and NH_4^+

The third set of treatments evaluated the trends of ammonia and nitrate together in the same experimental unit. NH_4^+ decrease and NO_3^- increase were different at all concentration levels evaluated (Tukey Kramer HSD, q=2.35, p=0.05, df=14).

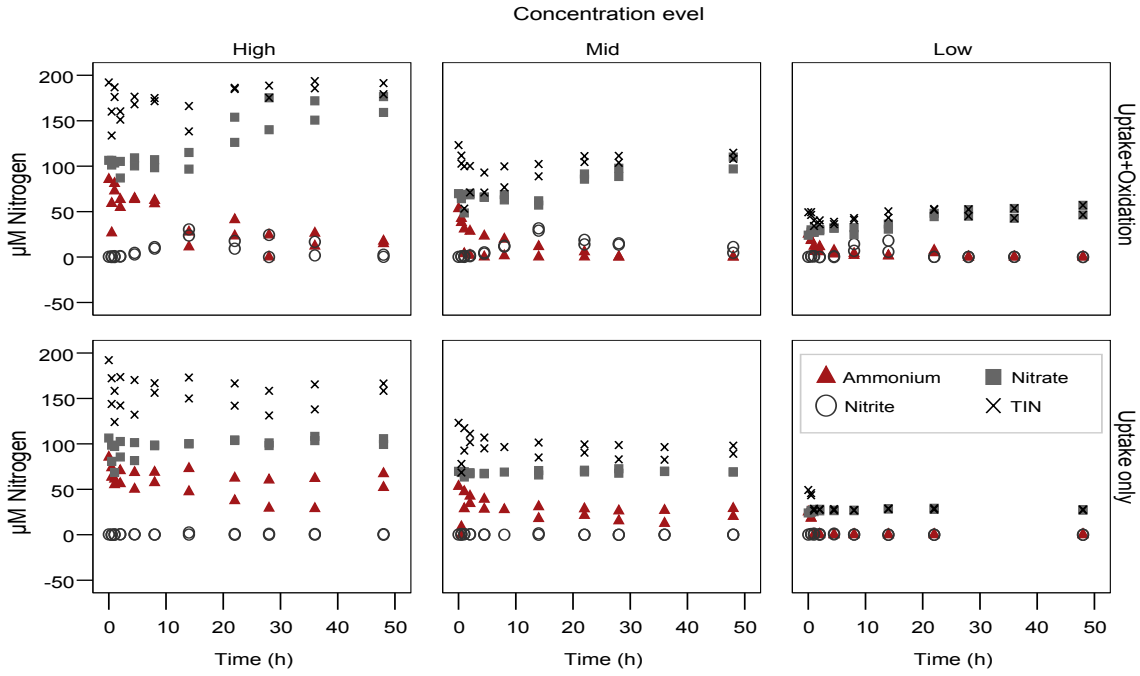


Figure 2.7 - Changes in ammonium, nitrite and nitrate in treatments with NO_3^-+NH_4^+. Acetylene inhibition is represented in *Uptake only* graphs (lower panels), and different concentrations are in different columns. Nitrogen mass addition by treatment is described in Table 2.1.

Nitrite production with acetylene inhibition was only 6% of the NO_2^- produced in the *Uptake+Oxidation* treatments. (Figure 2.7, upper panel). In the *Uptake* treatments, TIN and ammonium decreased with a similar pattern and nitrate was relatively stable without net increase of NO_3^- by the end of the experiment. The TIN in the *Uptake+Oxidation* reflected the initial NH_4^+ decrease and later NO_3^- increase; however, initial and final TIN mass was almost the same (Figure 2.7, lower panel).

Ammonium and nitrate uptake velocity

Ammonium and nitrate uptake was modeled with a zero order reaction to calculate the uptake velocity *V*. The uptake velocity (in μmol h^{-1}) is assumed to be the slope of the linear regression of the concentration (μm N) by time (Figure 2.8).

The ammonium uptake velocity in the treatments with ammonia oxidation blocked by acetylene was 0.97 μmol h^{-1} at low concentration (TAN_0=1.09 mg N L^{-1}, R^2=0.78) and 1.34 μmol h^{-1} at high concentration (TAN_0=4.7 N mg L^{-1}, R^2=0.65; Figure 2.10, left panel). These uptake velocities represent areal uptake rates estimated between 314 and 609 μmol N m^{-2} h^{-1} (Table 2.3).

Nitrate uptake velocities were lower than ammonium uptake velocities (Table 2.2. Figure 2.8, right panel). The nitrate uptake velocities were 0.15 μmol N h^{-1} at mid concentration ($NO_3^-_{init}$ = 3.4 mg N L^{-1}, R^2 = 0.55) and 0.17 μmol N h^{-1} at high concentration ($NO_3^-_{init}$ = 5.7 mg N L^{-1}, R^2=0.41). Nitrate uptake did not show any decrease at low concentration ($NO_3^-_{init}$ = 1.1 mg N L^{-1}) or when ammonium was present in the solution.

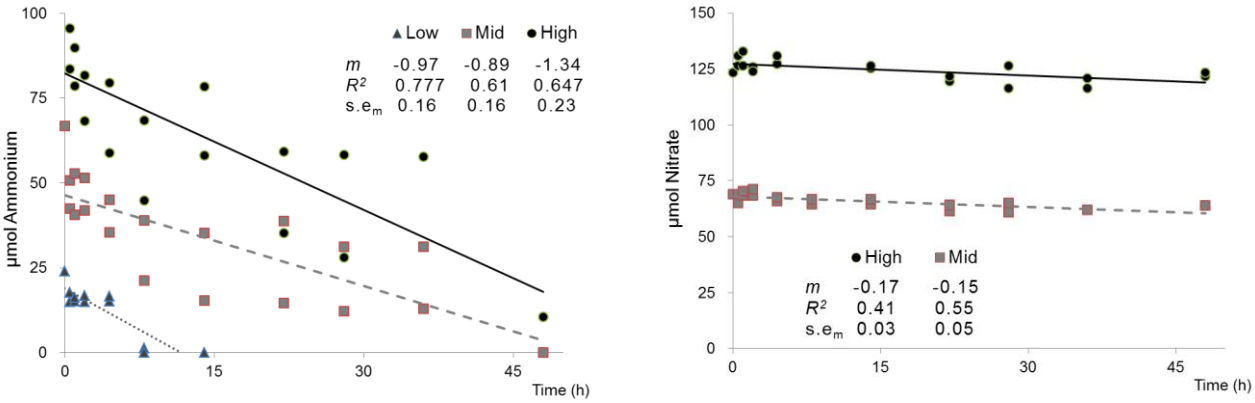


Figure 2.8 - Ammonium (*left*) and nitrate (*right*) uptake velocity at different concentrations. The slope of the linear regression estimates the uptake velocity *V* in µm N h^{-1}. Low concentration treatment not shown due to opposite trend (nitrate production). Nitrogen concentration level is described in Table 2.1.

The effect of light on NO_3^- and NH_4^+ assimilation

The experiment that evaluated the effect of light on nitrate and ammonium assimilation showed that nitrate decrease was significantly higher with light than in darkness (t = 1.98 p = 0.05, df=31). Despite that the observed decrease in ammonium with light was greater than in darkness, the difference was not quite significant (F = 3.23, p = 0.08, df=31; Figure 2.9). Table 2.3 shows the rates calculated for the experiment.

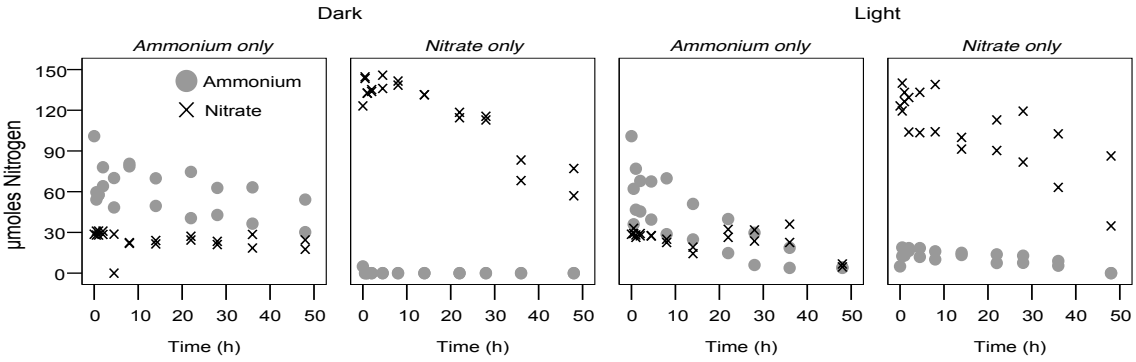


Figure 2.9 - The effect of light on ammonium and nitrate decrease (µmoles N) by time. Panels show high concentration treatments only (4.7 mgN-NH_4^+/L or 5.7 mgN-NO_3^-/L)

Table 2.2 – Calculated uptake velocity (V in μmoles N h^{-1}), areal uptake rates (μmoles and mg N m^{-2} h^{-1}) and biomass-weighted uptake rates (μg N per g $biomass^{-1}$ h^{-1}) of ammonium and nitrate evaluated in light and dark conditions in ceramic tiles. N species represent the available nitrogenous form for uptake in each experimental unit. Concentration levels low (1.09 mgN-NH_4^+/L and 1.07 mgN-NO_3^-/L) and high (4.71 mgN-NH_4^+/L and 5.75 mgN-NO_3^-/L). Numbers in brackets represent the standard error of uptake velocity ($s.e._v$); intervals for rates were calculated by propagation of $s.e_v$. Estimated biomass in tiles was 73.6 ± 37.2 g (AFDW) m^{-2}. ***** Represents nitrate production

Treatment	**Concentration level**		**Uptake velocity (μmol h^{-1})**	**Uptake rate (μmol N m^{-2} h^{-1})**	**Uptake rate (mgN m^{-2} h^{-1})**	**Uptake rate (*μ*gN g $biomass^{-1}$ h^{-1})**
Ammonium only	*Low*		0.97 *(0.16)*	314 - 438	4.4 - 6.1	57 - 79
	Mid		0.89 *(0.16)*	283 - 407	4 - 5.7	51 - 74
	High		1.34 *(0.23)*	430 - 609	6 - 8.5	78 - 110
Nitrate only	*Low*		*n.a.*	*n.a.*	*n.a.*	*n.a.*
	Mid		0.15 *(0.03)*	47 - 70	0.7 - 1	8 - 13
	High		0.17 *(0.05)*	47 - 85	0.7 - 1.2	8 - 15
Ammonium+Nitrate	*Low*	NH_4^+	0.51 *(0.27)*	92 - 302	1.3 - 4.2	17 - 55
		NO_3^-	*-0.03 (0.2)*	*4-19 **	*0.1 - 0.3 **	*1 - 4 **
	Mid	NH_4^+	0.55 *(0.13)*	163 - 364	2.3 - 3.7	20 - 48
		NO_3^-	*-0.04 (0.02)*	*6 - 25 **	*0.1 - 0.4 **	*1 - 5 **
	High	NH_4^+	0.88 *(0.24)*	248 - 434	3.5 - 6.1	45 - 78
		NO_3^-	*- 0.18 (0.1)*	*31 - 109 **	*0.4 - 1.5 **	*6 - 20 **

Table 2.3– Calculated uptake velocity (V in µmoles N h^{-1}), areal uptake rates (µmoles and mg N m^{-2} h^{-1}) and biomass-weighted uptake rates (µg N per g $biomass^{-1}$ h^{-1}) of ammonium and nitrate evaluated in light and dark conditions in a full factorial experiment 2^3. N species represent the available nitrogenous form for uptake in each experimental unit. Concentration levels low (1.09 mgN-NH_4^+/L and 1.07 mgN-NO_3^-/L) and high (4.71 mgN-NH_4^+/L and 5.75 mgN-NO_3^-/L). Numbers in brackets represent the standard error of uptake velocity ($s.e._v$); intervals for rates were calculated by propagation of $s.e_v$. Estimated biomass in tiles was 73.6 ± 37.2 g (AFDW) m^{-2}.

N specie	Concentration level	Condition	Uptake velocity (µmol h^{-1})	Uptake rate (µmol N m^{-2} h^{-1})	Uptake rate (mgN m^{-2} h^{-1})	Uptake rate (µgN g $biomass^{-1}$ h^{-1})
NH_4^+	High	Light	1.34 (0.2)	443 - 598	6.2 - 8.4	80 - 108
		Dark	0.55 (0.12)	165 - 358	2.3 - 3.6	30 - 47
	Low	Light	1.04 (0.24)	74 - 260	1.0 - 3.6	13 - 47
		Dark	0.85 (0.39)	44 - 346	0.6 - 4.8	8 - 63
NO_3^-	High	Light	1.5 (0.15)	523 - 639	7.3 - 8.9	94 - 115
		Dark	1.26 (0.04)	472 - 503	6.6 - 7.0	85 - 91
	Low	Light	0.64 (0.24)	154 - 340	2.2 - 4.8	28 - 61
		Dark	0.41 (0,06)	138 - 185	1.9 - 2.6	25 - 33

DISCUSSION

Epilithon contribution to nitrogen cycling downstream of the KTP

In general, the expected trends in the conceptual model presented in Figure 2.1 were corroborated in the experiments. When ammonium was the only N source, TIN decreased in the *Uptake* treatments and remained stable in *Uptake+Oxidation* treatments. With nitrate as only N source, TIN decreased in the treatments with mid and high initial concentration, but showed a slight increase in the low NO_3^- concentration treatment, possibly a result of internal NH_4^+ regeneration. Finally, in the treatment with NO_3^-+NH_4^+, the TIN varied little in all experimental conditions (see Figure 2.6)

The epilithic community established on the ceramic tiles assimilated ammonium at rates above 300 μmoles N-NH_4^+ m^{-2} h^{-1}, whereas nitrate was assimilated at a much lower rate (< 70 μmoles N-NO_3^- m^{-2} h^{-1}). With the estimated uptake rates obtained in these experiments, ammonium assimilation by epilithon represented net N removal between 4.8 and 7.3 mg N-NH_4^+ m^{-2} h^{-1} for the 48 h duration of the experiments, whereas nitrate assimilation accounted for net removal between 0.8 and 0.9 mg N-NO_3^- m^{-2} h^{-1}. The rate of ammonium uptake estimated for the Grand River is much higher than the rates measured by Biggs (1990) between 0.24 and 1.6 μmol N-NH_4^+ m^{-2} h^{-1} in a New Zealand stream at concentrations of approximately 0.4 mg N-NH_4^+/L. The estimated nitrate uptake rate for the Grand River is at the low end of the rates reported by Mulholland *et al*. (2004); between 82 and 424 μmol N-NH_4^+ m^{-2} h^{-1}. He reported higher rates in a first order stream enriched with nitrate to a concentration of 1.65 mgN-NO_3^-/L, thus it is possible that an increase in NH_4^+ concentration would result in increased uptake rates in the Grand River.

Assuming that similar uptake rates occurred in the Central Grand River downstream of the KTP effluent, and adjusting for the biomass estimated in the field (8.8-29.2 g biomass AFDW m^{-2}), ammonium uptake by the epilithic community in the Central Grand River would have been between 46 and 153 μmoles N-NH_4^+ m^{-2} h^{-1} and the nitrate uptake approximately from 8 to 26 μmoles N-NO_3^- m^{-2} h^{-1} during the day. Ammonium uptake at this rate upstream of the KTP (where the average biomass was almost double that downstream of the KTP) could have resulted in the low ammonium concentration observed above the KTP effluent (0.08 ±0.1 mgN-

NH_4^+/L at the Victoria St. sampling location); thus the influence of the Waterloo wastewater treatment plant effluent was not observed any longer by this point. The annual average total phosphorus in 2013 was similar upstream (0.07 mg TP/L) and downstream (0.06 mg TP/L) of the KTP, similar to soluble reactive phosphorus (0.033 mg SRP/L upstream and 0.041 mg SRP/L downstream); thus, the greater observed biomass upstream of the KTP effluent might be explained by additional factors (see below, *Periphyton biomass estimates*). Low macrophyte biomass downstream of the KTP was observed by Hood (2012) and she suggested that ammonium toxicity may be involved.

Acetylene inhibition allowed the isolation of ammonium uptake from nitrification in the epilithic community. Acetylene has been used to simultaneously block nitrification and denitrification (Teissier and Torre 2002); therefore, nitrate decrease is expected solely from uptake. Organic matter mineralization was not expected to be an important factor during the experiment due to its short duration and the relative abundant nutrient supply.

The observed pattern of NH_4^+ decrease in the *Uptake+Oxidation* treatments was similar to the nitrification activity of aerobic ammonia oxidizers in sediments reported by Henriksen (1980) and Sonthiphand *et al*. (2013). The ammonium removal in experimental conditions was between 100% and 26% of the initial ammonium mass in each experimental unit. In the field, there are additional factors affecting the ammonium decrease from the water column, such as dilution, nitrification, volatilization and sorption into sediments. The total contribution of epilithon to ammonium decreases in different ecosystems has been estimated at 18% in a river receiving WTP discharge in Japan (Ogura et al. 2009) to as much as 66% of the total nitrogen removal in grasslands streams (Simon *et al*. 2007). Thus, by estimating the ammonium assimilation (blocking nitrification) in epilithon of the Grand River and comparing it with previous studies, it is evident that epilithon have an important role in the nitrogen cycling in rivers within heavily urbanized areas.

High NH_4^+ loads from the KTP before upgrades may have caused the biofilm to use less nitrate given the energetically favourable assimilation of ammonium. The experimentally-obtained biomass-weighted nitrate uptake rates were around 12 µg $N\text{-}NO_3^-$ g $epilithon^{-1}$ h^{-1}. Previously,

gross nitrogen uptake by macrophytes with attached periphyton in different sections of the Grand River was estimated as from 42 to 100 μg N per g macrophyte^{-1} h^{-1} (Hood 2012). The uptake of nitrate by epilithon in temperate forested streams was reported between 12 mgN-NO_3^- g biomass^{-1} h^{-1} (Triska et al 1985) to undetectable (Hamilton et al. 2001).

In these experiments, nitrate uptake by epilithon was small (< 25 μm N m^{-2} h^{-1}) or unmeasurable, and was observed at concentrations higher than 3.3 mgN-NO_3^-/L. Epilithon uptake velocities can be low compared to the uptake rates of macrophytes on an areal basis due to the large biomass of macrophytes (Sand-Jensen and Borum 1991). Sosiak (2002) reported that macrophyte biomass in the Bow River (Alberta) declined as a result of a reduction in nitrogen in wastewater effluent. The effluent discharged by the KTP may affect the biomass and growth of some macrophytes (Dennison et al. 1993). If the KTP effluent impacts the macrophytes, and the macrophytes represent an important substrate for periphyton, then the upgrades to the KTP will likely represent an impact to the macrophyte-periphyton association ultimately impacting their role in the nitrogen cycling in the Grand River.

The estimated uptake rates *in situ* extrapolated using the biomass measured in the river could be over- or underestimated. Some factors should be considered before drawing conclusion from the experimental data. For example, the tiles and the cobbles analyzed for biomass could have had different species composition. The large surface area and small volume in the experiment might have affected uptake rates (Nielsen and Jensen 1990). Vis et al. (2007) estimated that 16% of annual primary production in the Saint Lawrence River was due to epiphyton biomass (epilithon+periphyton), comprising an important fraction of the in-river productivity. Periphyton biomass in the Grand River could also account for a large part of the net primary productivity due to the abundant TIN in the water column as a result of the KTP discharges, in addition to the agricultural inputs in the upper part of the watershed.

Preferential uptake of ammonium over nitrate

Ammonium is considered to inhibit nitrate uptake at concentrations higher than 1 μmol N (Dortch 1990). In the following section, the term *ammonium preference* is used to refer to the indirect interaction between nitrogen species, given that inhibition implies a direct interaction

between them. In a thorough review, Dortch (1990) suggested that the mechanism of preference of one nitrogen species over the other is not simply either inhibition or preference, but rather a combination of processes affected by concentration and environmental conditions (pH, temperature and redox conditions). To quantify ammonium or nitrate preference, Dortch suggested relating the uptake ratios (NO_3^-/TN, NO_3^-/NH_4^+ or NO_3^+ uptake) in presence or absence of ammonium. She found a wide range of ratios, including nitrate uptake higher than ammonium uptake in some cases. she also found that changes in environment nitrogen concentrations do not necessarily result in changes in uptake rates unless the system is closed and enough time is allowed to leave the systems to re-establish equilibrium. She developed and index, the rate of preferential incorporation (RPI):

$$RPI_{NO_3^-} = \frac{P_{NO_3^-} / \Sigma\rho_N}{[NO_3^-] / [\Sigma N]}$$

Where P_{NO3}^- is nitrate uptake rate, $\Sigma\rho_N$ is the sum of uptake rates of all nitrogen species, $[NO_3^-]$ nitrate concentration and [ΣN] is the sum of all nitrogen concentrations. The equation is equally valid when TAN is used instead of NO_3^- (Takahashi and Saijo 1981). Values less than 1 indicate a preference for ammonium. Using this equation with the experimental data resulted in a RPI between 0.04 and 0.17, thus supporting the hypothesis that, in this experiment, ammonium was preferred over nitrate. Preference for ammonium is not universal but appears to be common because of NH_4^+ is reduced and therefore energetically advantageous. After recent upgrades to the KTP, the greater nitrate concentrations and availability dowstream of the KTP may promote greater nitrate assimilation by macrophytes and periphyton downstream.

The effect of light on nitrate uptake

In this experiment, the ammonium uptake at high concentration was not significantly different in continuous illumination or in darkness, in contrast to a study by Lipschultz et al (1985), who found that ammonium oxidation responded to light levels and ammonium uptake by phytoplankton increased with light intensity. Nitrogen assimilation and incorporation requires more metabolic energy for nitrate than for ammonium. Preference for ammonium has been observed in terrestrial plants (Gunnison and Barko, 1988; Lambers et al., 1998) and bacteria (Henriksen 1980). Nitrate assimilation requires H^+ ions, whereas ammonium assimilation

(NH_4^+) produces H^+ (Raven 1984); therefore, when both NH_4^+ and NO_3^- are present, ammonium uptake likely occurs first, followed by nitrate uptake once ammonium is consumed.

The experiment in light and dark conditions provided supporting information that nitrate uptake is light-dependant, given that the enzyme nitrate reductase (Lambers et al. 1998) requires energy (Hageman and Flesher 1960). Nitrate uptake typically occurs at high-nitrate, low-ammonium concentrations (Cedergreen and Vindbæk (2003); however, it is possible that the 48 h of these experiments was not enough time to observe more intense nitrate assimilation by the epilithic community.

Co-dependence of nutrient status during DIN uptake

It has been estimated that 50% of the carbon fixed by algae is coupled to nitrogen uptake, given that assimilation of nitrogen into proteins requires photosynthetically-derived energy and a carbon backbone. In epilithic communities, where bacteria and algae are closely associated, algae fix carbon and bacteria could mineralize organic matter into nutrients such as ammonium (Böckelmann et al. 2000; Araya et al. 2003), thus leading to an increase in nitrate even when nitrate was the only source of nitrogen supplied to the experimental.

The treatments that evaluated NO_3^-+NH_4^+ represented the conditions commonly observed in the Grand River, particularly before upgrades, when ammonium was discharged in high concentrations. The NO_3^-/ NH_4^+ mass ratio in these experiments were similar across treatments (low=0.96; mid=1.3 and high=1.25); and the NO_3^-/ NH_4^+ ratios observed in the Grand River close to the KTP effluent were between 1 and 3 in the summer of 2010 to 2012. Therefore, the uptake velocities and the trends reported in this research are a good representation of what occurred in the Central Grand River downstream of the wastewater treatment plants before upgrades.

Dissolved organic nitrogen (DON) was not measured. However, previously collected water samples in the central Grand River downstream of the KTP (2011-2012) had an average DON of 1.2 (±0.6) mg N/L, comprising between 12 and 25% of the TN measured in that reach of the river. It is possible that DON mineralization is an additional ammonium source. The average DOC in this same section of the river was 7.2 (±1.7) mg C/L.

Initial total phosphorus (TP) and soluble reactive phosphorus (SRP) in this experiment were 25 and 3 μg P/L, respectively, so the Redfield ratios for the treatments were between 90 and 300, consistent with represent P limitation. Barlow-Busch et al. (2006) reported epilithon+seston uptake rates from 0.06 to 1.83 μg P cm^{-2} hr^{-1} (9.6 to 590 μm P m^{-2} h^{-1}), with seston more active than epilithon per unit biomass. Even with P in excess downstream of the KTP, Morrison suggested that the biomass downstream of the WTP's in the Grand River did not assimilate P to rates that could represent a net sink for the observed P concentration (i.e. low P retention); therefore, simultaneous N and P uptake in the Central Grand River could be biomass limited.

Periphyton biomass estimates

The average biomass estimated on the tiles was 73 g/m^2 (AFDW), whereas the biomass estimated from cobbles in the Grand River was less than half (8 to 29 g/m^2), arguably due to different growth and loss conditions. It has been reported that diatom-dominated mats have lower biomass than cyanobacteria-dominated mats (Azim and Asaeda, 2005). The chl*a*:biomass ratio (0.01%) and the autotrophic index (AI=[AFDM/chl*a] = 1400*) suggested that the epilithon downstream of the KTP effluent was probably composed by Bacillarophyceae, Cyanobacteria and a heterotrophic community. This taxonomical composition would be in agreement with Chételat et al. (1998) for rivers in Eastern Canada. Also, the color of the colonized tiles (brown-green filamentous crusts, following Azim et al. 2005) support the presence of Cyanobacteria, Chlorophyta and diatoms (Bacillarophyceae). A previous study in the Grand River showed seasonal differences in presence and dominance of diatoms, and reported that the epilithon in the Central Grand River was dominated by species of the genera *Achnanthes, Cocconeis, Gomphonema, Naviculla* and *Rhoicosphenia* (Rott et al. 1998). These epilithic communities grow in shaded, shallow habitats with slow flow and moderate light, usually with limited photosynthetic rates but rapid growth and high diversity (Azim et al. 2005).

The cobbles were exposed to variable flow conditions for a longer period, whereas the tiles represent biomass accrual for a short period under more favourable conditions (fewer oscillations in flow regime and no shade). These different conditions could have led to different community composition, thus different uptake rates could be expected when assessing epilithic

biomass in different stages of colonization and taxonomic composition. Disturbance events such as flow augmentation, enhanced sedimentation and dislodgement during spring melt involves longer recovery time, and the re-colonization of hard surfaces by periphyton depends on the floating material, floccules and cohesive material available at a given location. Therefore, the biomass on cobbles may have been subjected to variable flow conditions, at least during one growing season, ranging from spring melt to summer storm. The biomass growth on tiles might represent species that rapidly colonize available surfaces when flows are relatively stable. Grazing and scouring were not evaluated in this research; nevertheless, net periphyton accumulation in the Grand River was estimated to be as high as 318 mg m^{-2} day^{-1} ash-free weight (accumulation minus loss; Liaw and Maccrimmon 1978).

CONCLUSIONS

Acetylene treatments blocked bacterial ammonia oxidation, thus ammonium decrease due to assimilation could be estimated. Based on the rates obtained in these experiments, the epilithon on ceramic tiles downstream of the KTP effluent preferred ammonium over nitrate, assimilating ammonium in experimental conditions at velocities between 377 and 519 μmol N m^{-2} h^{-1}. Nitrate uptake had a lower uptake velocity and sometimes was produced instead of being consumed. The fastest nitrate uptake velocities were 58 to 65 μmol N m^{-2} h^{-1}. Light had a significantly different effect on nitrate uptake, but the effect of light on ammonium uptake was not quite statisitically significant. Epilithon uptake was responsible for 26% to 100% of the ammonium loss in these experiments, suggesting that assimilation and nitrification by the attached periphytic community both played a significant role in the decrease of ammonium downstream of the largest WWTP on the Central Grand River, Ontario.

CHAPTER 3 - NITROGEN ISOTOPIC FRACTIONATION DUE TO AMMONIA VOLATILIZATION IN CONTROLLED CONDITIONS.

INTRODUCTION

Ammonia (NH_3) is a ubiquitous molecule in the environment. It is commonly released from decaying biomass, it is constantly formed and removed a result of microbial activity and added to the soil as fertilizer (Freney *et al*., 1983). It is a molecule that dissolves into water and easily volatilized to the gaseous phase, also susceptible to adsorption onto clays (Environment Canada 1984). As a gas at normal temperature and pressure, ammonia will volatilize from soil, fertilizers and manure; however, it could react with protons and acidic compounds to form ions, resulting in storage of ammonium (NH_4^+) in solution or in salts (Freney *et al*., 1983). Ammonium is the conjugate acid of ammonia (NH_3) in solution, whose equilibrium [$NH_3+H_2O \leftrightarrows NH_4^+ + OH^-$] depends on mainly on pH (Figure 3.1) and temperature.

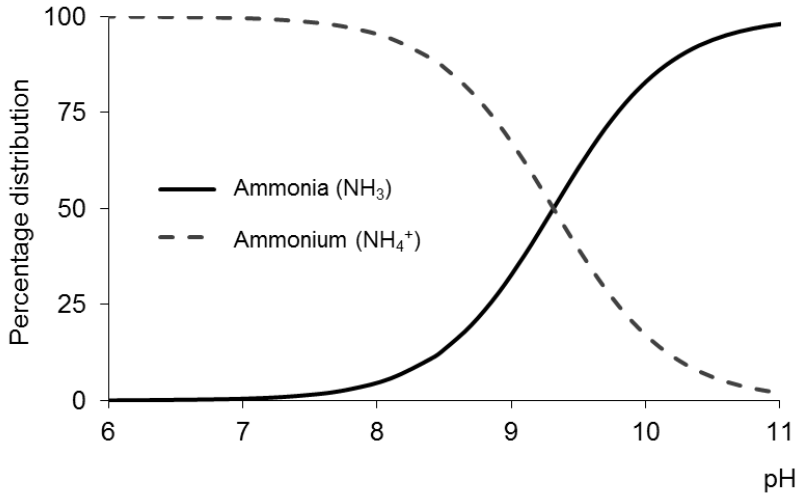


Figure 3.1 - Effect of pH on the NH_3–NH_4^+ equilibrium in solution at 296 K (23ºC).

The proportion of each of the Total ammonia nitrogen species (NH_3 or NH_4^+) in aqueous solutions depends solely on TAN concentration, pH and temperature. Ionic strength is also relevant in hard waters or saline systems, but in most freshwaters (up to 300 mg L^{-1} dissolved solids) its effect is considered negligible.

Emerson et al. (1975) evaluated various temperature-dependency relationships solution of ammonium and found that a two-parameter fit ($pK=A+B/T$) was adequate to represent the

dependency of ammonia pK_a on temperature. The ammonia pK_a (the acid dissociation constant) can be calculated for any solution which temperature is between 0 and 30ºC and pH from 6.0 to 10.0 as follows

$$pK_a = 0.09018 + \frac{2729.92}{T} \quad . \qquad \textit{Equation 1}$$

In order to express the fraction of unionized ammonia in an aqueous solution using the calculated pK_a value from equation 1, the rearranged equation is as follows:

$$f = \frac{1}{(10^{pKa-pH} + 1)} \qquad \textit{Equation 2}$$

Lastly, the concentration of dissolved NH_3 in the aqueous solution is obtained from equations 1 and 2:

$$[NH_3] = TAN \times f \qquad \textit{Equation 3}$$

Thus, the volatilization of $NH_{3(g)}$ is related to pH as a result of the ammonium-ammonia ionization and to temperature by Henry's law constant ($K_H=p_a/C_w$) given that the rate of NH_3 volatilization is a function of the concentration of the gas in the atmosphere (*i.e,* its partial pressure p_a), its concentration in equilibrium with the aqueous phase (C_w) and its mass transfer coefficient (Sommers 1985). Ammonia behaves different to other gases due to its high solubility in water, which means that the aqueous-gas boundary layer control the overall transfer velocity, thus influencing the $NH_4^+ \leftrightarrows NH_3$ equilibrium (Finlayson-Pitts and Pitts 1986).

Isotopic fractionation during ammonia volatilization has been studied in plants leaves (Farquhar et al 1983) and experimental conditions at variable pH conditions (Kirshenbaum 1947, Li et al 2012), reporting variable isotopic fractionation factors during the overall ammonia volatilization process. To my knowledge, there are no estimates of the isotopic fractionation associated with ammonia volatilization in a river impacted with wastewater discharges. Measuring the $\delta^{15}N_{NH_4^+}$ due to volatilization provides a quantitative identification of the ammonia volatilization in rivers (Robinson 2001), which assists in the quantification of N loss from aquatic systems impacted by human activities.

Field measurements are complicated due to the continuous replenishment of the ammonia pool in the river water and the continuous changes in air velocity. Those field conditions would entail large variability of the results obtained on the field; thus, a series of experiments with solutions at different pH values were developed to measure the observed isotopic fractionation factor and estimate the kinetic and equilibrium isotopic fractionation factors.

Isotopic fractionation

The distribution of isotopes between two reservoirs or substances can be described by Rayleigh equations. One type of Rayleigh fractionation entails three conditions: *i)* the material is continuously removed from a reservoir (*substrate*), *ii)* the isotopic fractionation associated with the removal of material is described by the isotopic fractionation factor α , and *iii)* the isotopic fractionation factor is the same during the process (Kendall and Caldwell 1998).

Isotopic fractionation occurring under open- or closed-systems conditions are distinguishable by the curves generated by the substrate and the product. In an open system, the substrate is continuously removed from the reservoir under conditions of a constant fractionation factor (Kendall and Caldwell 1998). An example of this system is the evaporation of water from a lake. A closed system is defined when the system has two reservoirs and the material is moved from one reservoir to another and isotopic equilibrium is maintained throughout the process. An example of a closed system fractionation is the condensation of vapor into droplets in a cloud (Kendall and Caldwell 1998).

Open and closed systems exhibit different isotopic fractionation patterns. For the equilibrium evaporation of water, the distillation observed is depicted in Figure 3.2. The solid and dashed curves represent water evaporation under open and closed systems respectively. The solid line A represents the $\delta^{18}O$ of the remaining water, which becomes heavier as the cumulative vapour (line C) is removed. If all substrate is converted into product, the final isotope delta of line C must be equal to the initial value of the line A. The line B represents the vapour formed instantaneously, and follows the same trend as line A, but with a lower $\delta^{18}O$ value. All solid lines (A-C) describe the behaviour observed during open-system evaporation under equilibrium conditions. In open-systems, when the transfer of mass is incomplete, the isotopic fractionation

ε generates a wide, yet predictable range in the isotope delta of the remaining substrate given that in very few occasions is all mas transferred from one reservoir to the other.

On the other hand, the dashed curves D and E represent water and vapour (respectively) during equilibrium evaporation in a closed system. The two phases (vapour and water) are in contact at all times, *i.e* they are in isotopic equilibrium; hence the isotopic mass is maintained throughout the process and results in parallel lines.

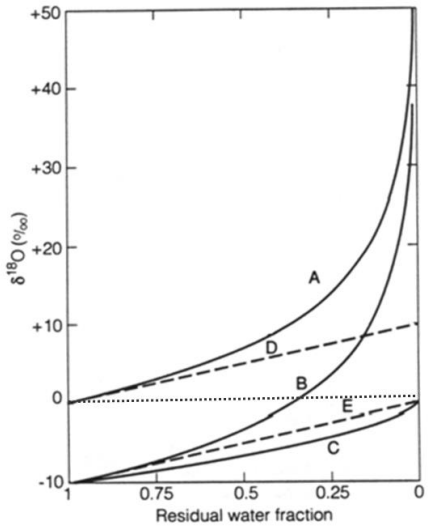


Figure 3.2 – Isotopic delta changes of $\delta^{18}O$ in open-system (*solid lines*) and closed system (*dashed lines*) during water evaporation. Open system: *A* represents the substrate water, *B* represents the instantaneous vapour and *C* is the cumulative vapour. Closed system: *D* and *E* represent water and vapour respectively. Taken from Kendall and Caldwell 1998.

Similar to the isotopic fractionation of water in the atmosphere, there is more than one step in which isotopic fractionation during ammonia volatilization occurs. Isotopic fractionation occurs due to the variable removal of ^{15}N respect to ^{14}N from the liquid phase into the gaseous phase (Kendall and Caldwell 1998). The observed isotopic fractionation factor between TAN and ammonia gas $[\alpha_{observed}\,(^{15}N/^{14}N)_{TAN/NH3(g)}]$ represents the measured difference between the isotope deltas of the substrate (TAN) and the product (NH_{3g}) as a result of the overall mass transfer in these experiments. The isotopic fractionation factor between the NH_4^+ and the $NH_{3(aq)}$ in the aqueous phase is the equilibrium isotopic fractionation factor $[\alpha_{eq}(^{15}N/^{14}N)_{NH4^+/NH3(aq)}]$, whereas the movement of ammonia from the aqueous phase (NH_{3aq})

to the gas phase (NH_{3g}) is the kinetic isotopic fractionation factor ($\alpha_{kin}(^{15}N/^{14}N)_{NH3(aq)/NH3g}$; Figure 3.3). For practical purposes, the isotopic fractionation factors above described will be referred to as $\alpha_{observed}$, α_{eq} and α_{kin} respectively. Loss of ammonia from soil or water is among the largest $^{15}N/^{14}N$ fractionating process reported in the literature (ε=40 to 60‰, Robinson 2001).

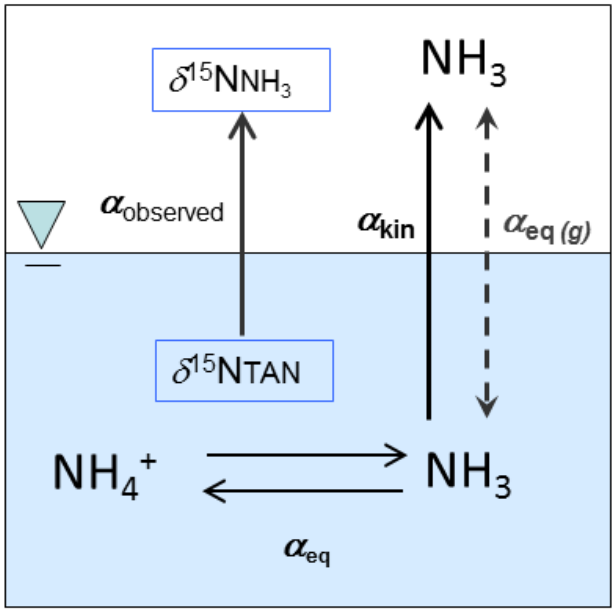


Figure 3.3 - Isotope fractionation factors (α) during the ammonia volatilization process. $\alpha_{observed}$ represents the isotopic fractionation factor measured in experimental conditions between total ammonia nitrogen (TAN) and volatilized $NH_{3g;}$ α_{eq} = equilibrium fractionation factor between ammonium (NH_4^+) and ammonia (NH_{3aq}); α_k = kinetic isotopic fractionation factor between NH_{3aq} and NH_{3g}. $\alpha_{eq(g)}$ is assumed to be zero due to continuous air movement between pools.

Measuring the nitrogen isotope fractionation factors in aquatic ecosystems entails experimental and analytical difficulties due to the mixing of pools, continuous replenishment of reservoirs, changes in temperature and flowing water. The objective of the series of experiments presented here is to assess the nitrogen isotope fractionation factors associated with ammonia volatilization at two pH values under controlled conditions and provide an analytical solution to estimate the equilibrium and kinetic isotope fractionation factors. These experiments measured the isotopic fractionation in an open system by removing the NH_{3gas} from the reservoir with continuous air flow and finally collecting the volatilized gas into an acid solution as NH_4^+.

MATERIALS AND METHODS

Two pH values were chosen for the experiments: pH 9.2 close to the pK_a of ammonia and pH 8.5, a value commonly measured in the Grand River. The experiment design allowed the observed isotopic fractionation factor (TAN→$NH_{3(g)}$) to be measured, whereas the equilibrium (α_{eq}) and kinetic (α_{kin}) isotopic fractionation factors were analytically obtained. The equilibrium fractionation factor of the ammonia gas between the aqueous phase and the gas phase ($\alpha_{eq(g)}$) was not measured nor calculated in these experiments given that the continuous air movement is assumed to prevent NH_{3g} re-invasion into the aqueous phase. The experiment at pH 9.2 was undertaken with a strongly buffered solution 0.025 M sodium tetraborate (9.5 g/L) whereas the experiment at pH 8.5 used Grand River water, a naturally buffered solution used in order to replicate the buffering conditions observed in the central Grand River . The river water filtered but not sterilized; thus, bacterial oxidation could have occurred in addition to volatilization. There were no pH changes observed during the experiment in the borate buffered solution (pH=9.2) or the river water (pH=8.5; measured with Hach HQ40d multi-parameter meter at experimental units t=0 h and t=600 h).

The experiments were conducted in two serum bottles, one containing a solution spiked with ammonium sulphate (*source*) to initial concentration greater than 20 mg N-NH_4^+/L (0.014 M). The second serum bottle (*trap*) contained an acidic solution in which volatilized ammonia was collected. The source and the trap bottles connected with plastic tubing are referred to as an experimental pair (Figure 3.4) measured in duplicates (n=2). All experimental pairs were sacrificed at different times (0 to 600 hours). The source serum bottles were 60 ml glass serum bottles, closed with grey butyl stoppers, containing 20 mL of the spiked solution, and continuous aeration from the bottom (air at 10 ml/min). Air was passed through glass wool to retain impurities. Air was used in the experiment given its accessibility to the experiment; no inert gas was used for the experiment due to the duration of the experiment (25 days). Preliminary test with acid trap blanks did not show any ammonium increase due to air bubbling. The acid traps were 40 ml serum bottles (tall serum bottles) with glass beads (2" height) to enhance liquid-gas interaction, which contained 20 ml of H_2SO_4-acidified water (pH≈4). Given the stickiness of the ammonia molecules, all glassware was washed in 0.1 M NaOH in order to reduce loss from the experimental pair. The ammonia stripped out from the

source unit was moved to the trap assuming unidirectional movement of the headspace. Ammonia was bubbled directly into the sulphuric acid aqueous column. Experiments were completed at room temperature (21-23°C), atmospheric pressure varied from 101.1 to 101.6 kPa during the 15 days of the experiment.

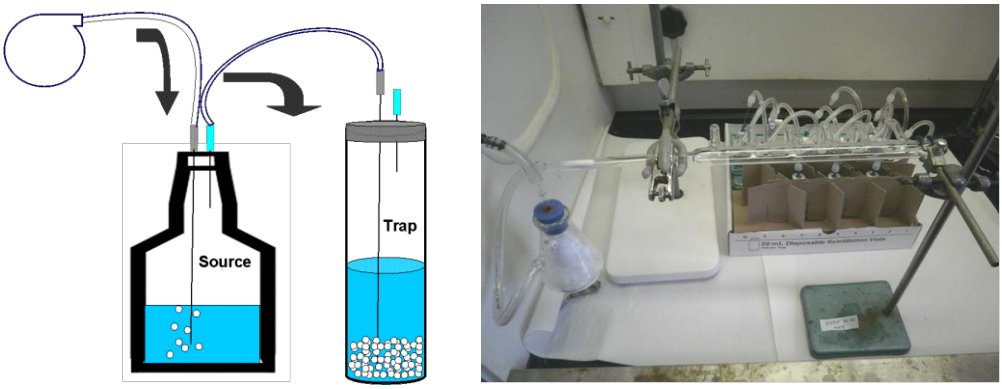


Figure 3.4 - Schematic diagram of the experimental pair (*left*) and photograph of experimental set-up (*right*). Air was allowed to pass through glass wool before entering the manifold. All tubing and valves were air tight.

In order to analytically measure TAN, the source solution was acidified with concentrated H_2SO_4 to pH≈4. 5 mL sub-samples from all sources and trap units were stored at 4°C until water chemistry analysis, the rest of the sample was frozen for isotopic analysis. TAN is the concentration of NH_3+NH_4^+, quantified by the standard colorimetric method (indophenol blue) whereas ammonia refers to the un-ionized form NH_3 and ammonium refers to the ion NH_4^+. TAN was measured by the colorimetric method blue indophenol using a Technicon AutoAnalyzer II (Technicon Instruments, USA). The detection limit was 0.05 mg N-NH_4^+/L.

Mass recovery was assessed as the percentage of the $TAN_{initial}$ mass measured at different times in each experimental pair (*source*+*trap* = 100%). A large aqueous column with glass beads and sulphuric acid in the trap unit achieved recovery efficiency of ≥ 90%.

To quantify the $\delta^{15}N_{TAN}$, an alkaline diffusion method was used. Following Spoelstra et al. (2006), a 4M KCl solution was prepared so that a total volume of 20 mL (standard or sample) with approximately 15 µg of nitrogen was deposited in 50 mL glass, Teflon-lined jars.

Diffusion traps were prepared by sealing an acidified (10 μL 0.2M H_2SO_4) quartz filter disk (Whatman QMA filters, baked at 550 ºC) in a section of polytetrafluoro-ethylene (PTFE) tape ('T-Tape'), in order to allow gas diffusion across the PTFE membrane. Each unit was taken to alkaline conditions (indicated by phenolphthalein indicator) by the addition of 0.2M sodium hydroxide (NaOH), buffered (pH~9.3) with 2 mL of $Na_2B_4O_7$ solution, and shaken continuously (80 rpm). After 10 days, PTFE traps were removed and the filter disks retrieved and placed in 4.5 ml glass vials; frozen and freeze dried. Disks were analysed with a Delta Plus, Continuous Flow Stable Isotope Ratio Mass Spectrometer (Thermo Finnigan/Bremen-Germany) coupled to a Carlo Erba Elemental Analyzer (CHNS-O EA1108 - Italy). Stable isotope ratios are expressed as delta (δ) in per mil (‰) relative to atmospheric N_2. Precision averaged ±0.3‰ for this analysis. Standards were prepared from IAEA 311 ammonium sulphate salts, characterized by the Environmental Isotope Laboratory – University of Waterloo using IAEA-N-1 (+0.43‰), IAEA-N-2 (+20.41‰) and IAES 305B (+39.8‰).

The experimental data were fit to the equation $\delta^{15}N_t = \varepsilon \ln f_t$; where $\delta^{15}N_t$ was experimentally obtained at different times *t*, with a correspondent fraction *f* of the $TAN_{initial}$. Then, the fractionation factor **ε** was obtained from a trial and error estimation of values from 18‰ to 32‰ (covering the range of commonly reported fractionation factors) and the fractionation factor ε assigned to each pH was the fractionation factor in closer agreement with the critical value of the goodness-of-fit test. The goodness-of-fit test was used to test the hypothesis than the observed frequency ($\delta^{15}N_t$) conforms to the expected distribution given by the fractionation factor ε. The *linest* function in Excel was used for calculating the standard error for the slope from the linear regressions of $log[f_{TAN}]$ *vs time*. The standard error for the *y* estimate was calculated from the linear regressions of *observed* $\delta^{15}N_{NH_4^+}$ – *modeled* $\delta^{15}N_{NH_4^+}$. A χ^2 test was done for the isotope mass balance for assess statistical differences between the observed and the expected distributions.

RESULTS

Total ammonia nitrogen mass recovery

Mass recovery was above 90% except for some units in the experiment at pH 9.2 (72 and 600 hours; Figure 3.5). The experiment completed with Grand River water had close to 100% mass

recovery at each time point. Low mass recovery is assumed to result from leakage (stoppers) or loss due to incomplete gas exchange at the acid trap.

Experimental rate constant k

The partition of TAN between the *source* and the *trap* is represented by a first order equation, with a rate constant k in hr^{-1} (Figure 3.6). The k value for each treatment is the slope of the linear regression log (f_{TAN})–time (best fit by least squares). Rate constants k for the experiment were $k_{9.2} = -2.8x10^{-3}$ h^{-1} ($\pm 0.42x10^{-3}$ s.e $_y$) for the experiment at pH=9.2 and $k_{8.5} = -0.6x10^{-3}$ hr^{-1} ($\pm 0.15x10^{-3}$ s.e $_y$) for the experiment at pH=8.5. Outlier experimental pairs with mass recovery evidently out of the trend (*i.e.*, mass recovery higher than the previous time, one at 600 h in each experiment) have been manually removed so they do not introduce noise to the curve fit. Here, outlier refers not specifically to erroneous data, rather address surprising veridical data. Due to the unexpected trend in mass recovery, the *identification/rejection* option was adopted. When outlier values are kept, the rate constants decrease, yet within the same order of magnitude ($10x^{-3}$). The curves without the outliers had better R^2 values.

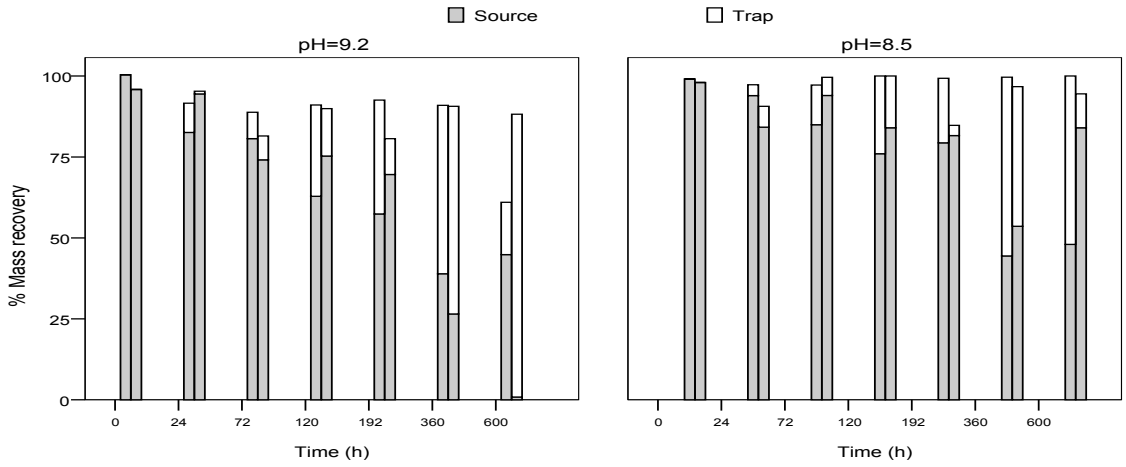


Figure 3.5 - Mass recovery of total ammonia nitrogen (in percentage) in the source-trap experimental set up. pH=9.2 treatment completed with a sodium tetraborate buffered solution; pH=8.5 treatment was conducted with Grand River water. Each bar represents one experimental unit.

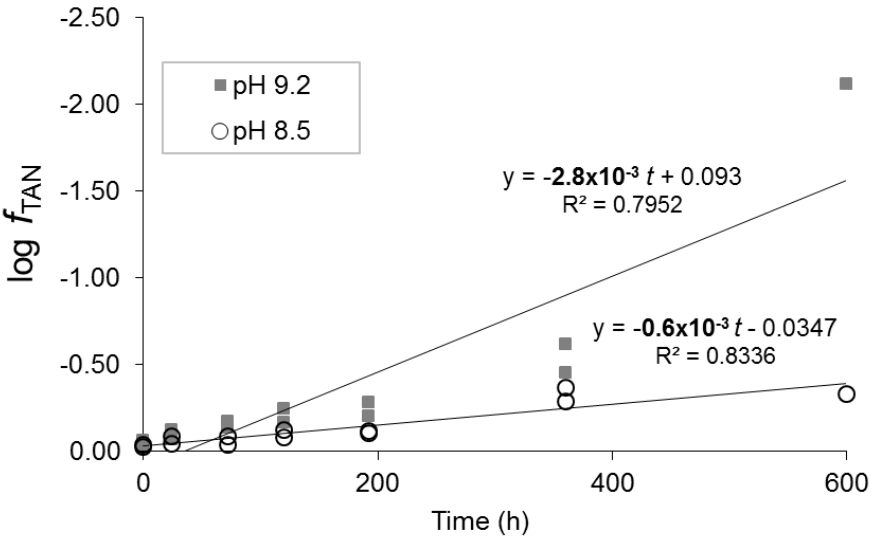


Figure 3.6 - Total ammonia nitrogen rate constant k (h^{-1}) obtained from the slope of a best fit line. TAN mass expressed as a fraction of initial TAN. $k_{8.5}$= -0.6x10^{-3} h^{-1} (±0.15 x10^{-3} s.e. of the estimate); $k_{9.2}$= -2.8 x10^{-3} h^{-1} (± 0.42 x10^{-3} s.e. of the estimate). One outlier replicate with mass recovery evidently out of the trend in each experiment have been manually removed (t=600 h).

Isotopic mass balance

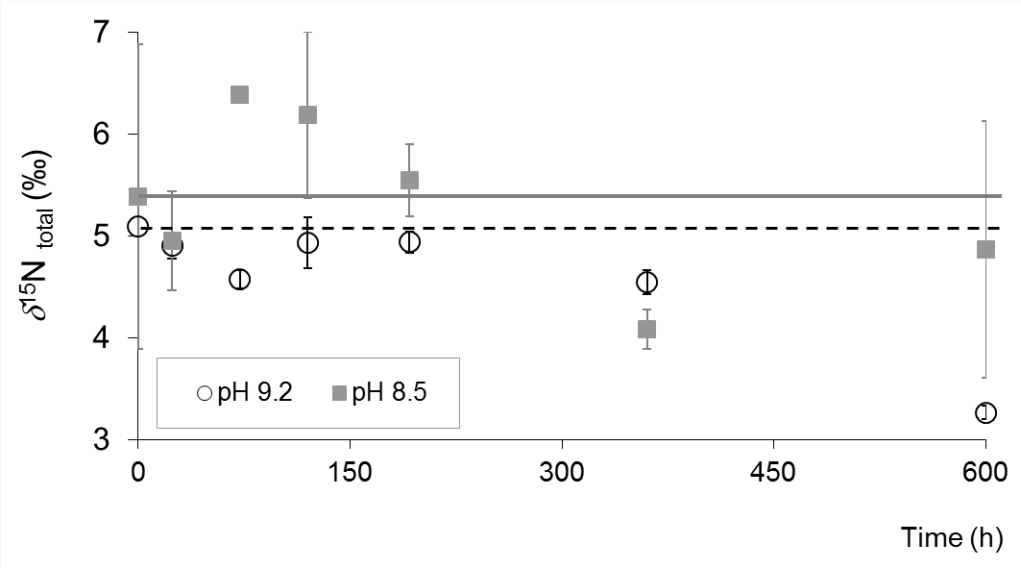


Figure 3.7 - Isotope mass balance of the total nitrogen expressed as $\delta^{15}N_{total}$ (‰) at different times during the ammonia volatilization experiment. Horizontal lines represent the initial $\delta^{15}N_{TAN}$: 5.4 ‰ at pH=8.5 (*solid line*) and 5.1‰ at pH=9.2 (*dashed line*). n=2, error bar ± 1.s.d.; precision averaged ±0.3‰ for $\delta^{15}N$ analysis.

The isotope mass balance for the experiments was done to account for all the nitrogen in the experiment (Figure 3.7).

$$m_{total} \times \delta^{15}N_{total} = m_{trap} \times \delta^{15}{}_{trap} + m_{source} \times \delta^{15}N_{source} \qquad \textit{Equation 4}$$

There was no statistical evidence of differences between the observed and expected distributions around the initial isotopic composition ($\delta^{15}N_{initial}$) of 5.1 ‰ at pH 9.2 ($\chi^2 = 12.48$) and 5.4 ‰ at pH 8.5 ($\chi^2 = 11.48$).

Observed isotopic fractionation during ammonia volatilization

The isotopic fractionation in both experiments followed a one-phase, open-system fractionation, thus by using a modification of the classical Rayleigh equation (following Mariotti et al. 1981), it is possible to fit the curve representing the isotopic fractionation of the *substrate* (source) undergoing volatilization in a finite supply reservoir:

$$\delta^{15}N_t = \varepsilon \ln f_t \qquad \textit{Equation 5}$$

Where $\delta^{15}N_t$ is the nitrogen isotope delta of the $TAN_{initial}$, the isotopic fractionation ε is the distribution of isotopes between the substrate and the product and f_t is the remnant fraction of substrate at time *t*. The *cumulative product* curve fit the experimental data following Mariotti et al. (1981) for the isotope delta of the product as a result of isotopic fractionation

$$\overline{\delta}_p = \delta_{S0} - \varepsilon_{p/s} \times \frac{f \ln f}{1 - f} \qquad \textit{Equation 6}.$$

Where $\overline{\delta}_p$ is the delta value of the cumulative product, δS_0 is the initial delta value of the substrate for the fraction *f* and $\varepsilon_{p/s}$ is the distribution of isotopes between the product and the substrate. Equations 5 and 6 were used to find the model fit to experimental data (curve fit obtained by sum of squares; Figure 3.8).

The $\delta^{15}N_{NH_4^+}$ below 0.2 of the residual ammonium at pH 9.2 were not trusted (clearly out of the trend), thus were not included in the model. The isotopic fractionation factors (from the relation $\varepsilon=\alpha-1$) that had the best fit (*goodness-of-fit* test) to the observed data were $\alpha_{9.2}=1.030 \pm 0.0025$

at pH 9.2 and $\alpha_{8.5}$=1.019 ± 0.0024 at pH 8.5 (± values represent standard error for the *y*-axis estimate).

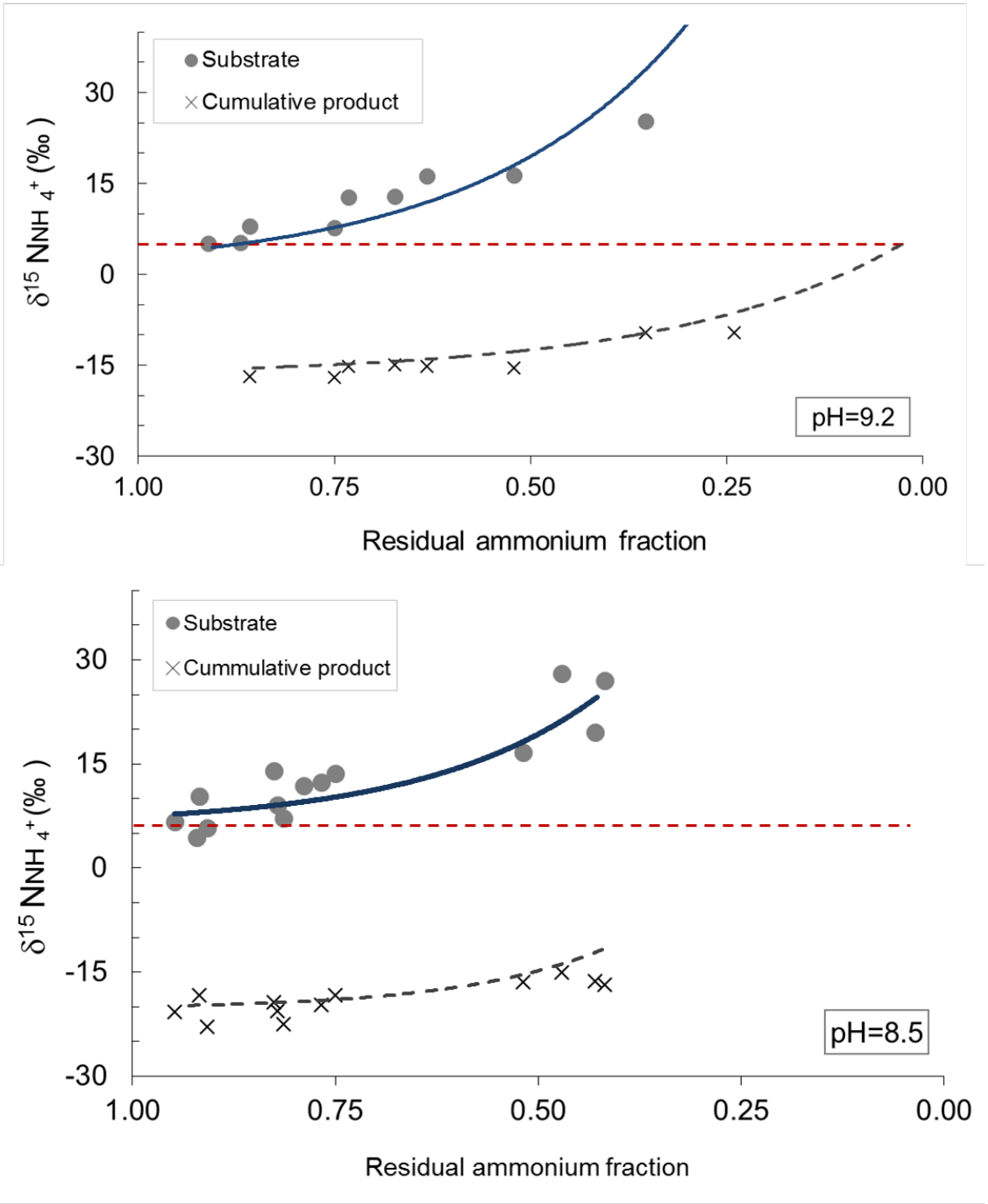


Figure 3.8 - Observed nitrogen isotope delta and model curves based on equations 5 (*substrate*) and 6 (*cumulative product*). Dotted line represents the initial $\delta^{15}N_{NH_4^+}$ (5.1‰ at pH 9.2, upper panel; 5.4‰ at pH 8.5, lower panel). Curves fit by sum of squares.

Calculated isotopic fractionation factors α_{eq} and α_{kin}

The ammonium isotope delta allows modelling the observed isotopic fractionation factor. The observed fractionation factor includes the equilibrium isotopic fractionation factor (α_{eq}) and the kinetic isotopic fractionation factor (α_{kin}); the former represents the equilibrium isotopic effect of the reversible reaction $NH_4^+ \leftrightarrows NH_3$, and the latter the kinetic fractionation during the flux from $NH_{3(aq)}$ to $NH_{3(g)}$.

From the mass balance

$$m_{TAN} \times R_{TAN} = m_{NH_3(aq)} \times R_{NH_3(aq)} + m_{NH_4^+(aq)} \times R_{NH_4^+(aq)} \qquad \textit{Equation 7}$$

where R is the ratio ${}^{15}N/{}^{14}N$; the isotopic ratio of TAN is:

$$R_{TAN} = \left[\frac{m_{NH_3(aq)}}{m_{TAN}} \times R_{NH_3(aq)}\right] + \left[\frac{m_{NH_4^+(aq)}}{m_{TAN}} \times \left(R_{NH_3(aq)} \times \alpha_{eq}\right)\right] \qquad \textit{Equation 8}$$

and the isotopic fractionation factor α_{eq} is the ratio of ratios:

$$\alpha_{eq} = \frac{R_{NH_4^+(aq)}}{R_{NH_3(aq)}} \qquad \text{or} \qquad R_{NH_4^+(aq)} = R_{NH_3(aq)} \times \alpha_{eq}. \qquad \textit{Equation 9}$$

Substituting equation 9 (as the ratio $R_{NH_4^+(aq)}$) in equation 8:

$$R_{TAN} = R_{NH_3(aq)}\left[\frac{m_{NH_3(aq)}}{m_{TAN}} + \frac{m_{NH_4^+(aq)}}{m_{TAN}} \times \alpha_{eq}\right]. \qquad \textit{Equation 10}$$

Similar to equation 9, the isotopic fractionation factor α_{kin} can be expressed as the ratio of ratios

$$\alpha_{kin} = \frac{R_{NH_3(g)}}{R_{NH_3(aq)}} \qquad \text{or} \qquad R_{NH_3(g)} = R_{NH_3(aq)} \times \alpha_{kin}. \qquad \textit{Equation 11}$$

Finally, equations 10 and 11 are substituted in the observed isotopic fractionation factor ratio of ratios $\alpha_{observed} = {R_{NH_3(g)}}/{R_{TAN}}$. Simplifying the equation is obtained the following

$$a_{obs} = \frac{\alpha_{kin}}{\frac{m_{NH_3(aq)}}{m_{TAN}} + \left(\frac{m_{NH_4^+(aq)}}{m_{TAN}} \times \alpha_{eq}\right)} \qquad \textit{Equation 12}$$

This equation can be solved when two pH values are experimentally evaluated. In this experiment, the $\alpha_{observed}$ for both pH values are known ($\alpha_{9.2}$=1.030 and $\alpha_{8.5}$=1.019) and the dissolved fractions of ammonium [$NH_4^+{}_{(aq)}$] and ammonia [$NH_{3(aq)}$] for each treatment are also known and can been made to remain constant through a pH-buffered experiment (Table 3.1).

Table 3.1 - Ammonia (NH_3) and ammonium (NH_4^+) expressed as mass fractions in the aqueous solution (*source*) at two different pH values. Mass fraction estimated from equations 1 to 3.

	$\frac{m_{NH_3(aq)}}{m_{TAN}}$	$\frac{m_{NH_4^+(aq)}}{m_{TAN}}$
pH=8.5	0.17	0.83
pH=9.2	0.47	0.53

Thus, equation 12 is expressed as a system of two equations:

$$1.030 = \frac{\alpha_{kin}}{0.47 + \left(0.53 \times \alpha_{eq}\right)} \quad (Eq.\ 13a) \qquad \text{and} \qquad 1.019 = \frac{\alpha_{kin}}{0.17 + \left(0.83 \times \alpha_{eq}\right)} \quad (Eq.13b)$$

The system of two equations is then solved by setting equations 13*a* and 13*b* equal:

$$1.030 \times \left[0.47 + \left(0.53 \times \alpha_{eq}\right)\right] = 1.019 \times \left[0.17 + \left(0.83 \times \alpha_{eq}\right)\right] \qquad \textit{Equation 14}$$

Thus, solving α_{eq}: $$\alpha_{eq} = \frac{1.030 \times \left(0.47 - 1.019\right) \times 0.17}{1.030 \times \left(0.53 - 1.019\right) \times 0.83} \quad ; \quad \boldsymbol{\alpha_{eq} = 1.036}$$

which is the $NH_4^+{}_{(aq)} \leftrightarrows NH_{3(aq)}$ equilibrium fractionation in the aqueous solution. Then, substituting α_{eq} in equation 12 to solve for α_{kin} with the fractions of NH_{3aq} and NH_4^+ at both pH values

$$\alpha_{kin8.5} = 1.030 \times \left[0.47 + \left(1.036 \times 0.53\right)\right] \quad ; \quad \boldsymbol{\alpha_{kin8.5} = 1.050}$$

$$\alpha_{kin9.2} = 1.019 \times \left[0.17 + \left(1.036 \times 0.83\right)\right] \quad ; \quad \boldsymbol{\alpha_{kin9.2} = 1.050}$$

which is the kinetic isotopic fractionation factor representing the ammonia gas escaping from the aqueous solution ($NH_{3(aq)} \rightarrow NH_{3(gas)}$).

The error for α_{eq} and α_{kin} was estimated by propagating the error ±0.0025 at pH 9.2 and ±0.0024 at pH 8.5 of the observed fractionation factors $\alpha_{observed}$. Thus, for these experiments α_{eq}=0.964

± 0.001 and α_{kin}=1.050 ± 0.003 (Figure 3.9). It is important to state that the error associated with pH measurements is not taken into account here.

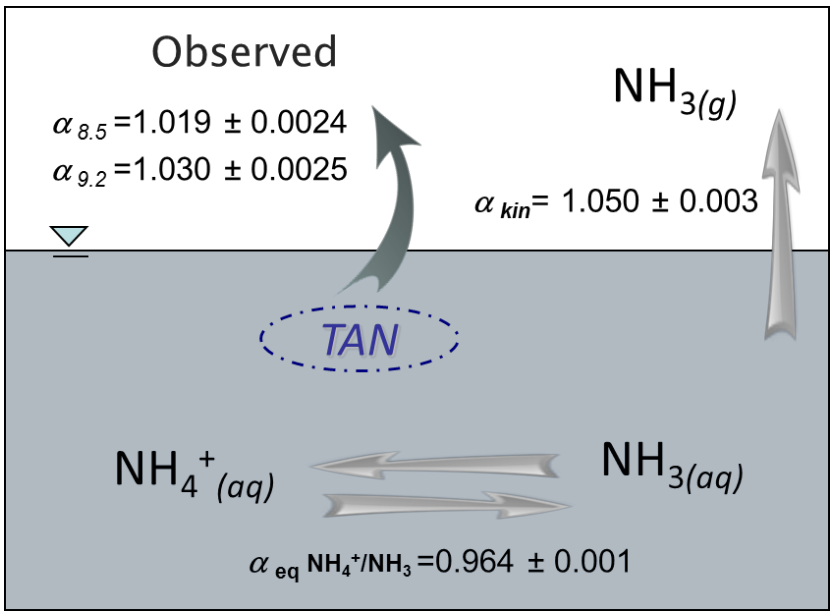


Figure 3.9 - Observed ($\alpha_{8.5}$ and $\alpha_{9.2}$) and calculated (α_{eq} and α_{kin}) nitrogen isotope fractionation factors for ammonia volatilization at two pH values (8.5 and 9.2). Isotopic fractionation factors (α) ± standard error of the estimate. Refer to Materials and methods for detailed experimental conditions.

DISCUSSION

Volatilization of ammonia involves two steps in which isotopic fractionation occurs and the combined effect of the observed equilibrium and kinetic isotope fractionation factor. The results presented allow calculating the fractionation factors associated with ammonia volatilization, and provide the equations necessary to calculate the equilibrium and kinetic fractionation factors in any aquatic system. This analytical solution can be used when the $\delta^{15}N_{NH_4^+}$ and the pH of the water is available.

Different approaches for estimating α_{eq} and α_{kin} have been previously reported and some of those results differ from the estimates here presented (Table 3.2). Some studies did not report initial and/or final pH values, thus equilibrium conditions were not clearly stated. However, changes in equilibrium conditions can be inferred from the description of the experiments.

Given the difficulty of estimating kinetic and equilibrium isotopic fractionation separately, much of the literature reported total fractionation. Urey *et al*. (1937) found a higher observed

isotopic fractionation factor as the concentration of dissolved ammonia increased and provided an average $\alpha_{observed}$ of 1.021 for solutions of saturated ammonium sulfate. Kirshenbaum *et al*. (1947) obtained the same increasing trend in the observed isotopic fractionation factor as ammonium mass increased. It is also possible that the observed fractionation factor was influenced by the purge rate (10 ml air/min) as the continuous removal of ammonia would require re-equilibration of the aqueous phase.

The kinetic isotopic fractionation factors reported by Farquhar et al (1983, α_{kin}=1.018) and Högberg (1997, α_{kin}=1.029) are considerably lower than the values estimated here α_{kin}=1.050. Kirshenbaum et al. (1947) reported variable observed isotopic fractionation by changing the pH in the solution. They also suggested that the kinetic isotopic fractionation factor α_{kin} at any TAN concentration can be estimated from the mass fraction of dissolved ammonia by the linear equation α= -0.029M+1.034; with *M* as the fraction of ammonia present in the aqueous solution. The graphical approach proposed by Kirshenbaum et al. (1947) resulted in an estimated isotopic kinetic fractionation factor α_{kin}=1.037, lower than the estimated in this experiment (1.050). Thus, using the ammonia mass fraction when experimental $\delta^{15}N_{NH_4^+}$ is not available should be used carefully. The analytical solution provided in equation 12 has the advantage of using experimentally obtained isotopic composition, so the isotopic fractionation factor can be modeled from experimental data.

Li *et al*. (2012) proposed modeling the equilibrium isotope fractionation factor between NH_4^+ and $NH_{3(aq)}$ in solution (α_{eq}) as follows:

$$10^3 \times \ln \alpha_{NH_4^+ - NH_{3(aq)}} = \frac{\delta^{15}N_{final} - \delta^{15}N_{initial}}{1 - f} \qquad \textit{Equation 14}$$

Table 3.2 - Ammonia isotope fractionation factors (α) experimentally obtained and estimated.

Reference	***Isotope fractionation factor***	***Reaction***	***What was measured of these variables***	***Comments***
Thode *et al.* (1945)	α1=1.031	$^{14}NH4^{+}_{(aq)} + 15NH3_{(g)} \leftrightarrows {}^{15}NH4^{+}_{(aq)} + {}^{14}NH3_{(g)}$	Isotope exchange factors through spectrograms, generated by ionization of gas samples. 180º focusing mass spectrometer, source-analyzer assembly; 0.3% precision.	Equilibrated liquid-gas ammonium solution at 25ºC. Gas samples measured by removing vapour from equilibrated systems and measured as N gas. Liquid samples were analyzed by removing solution and convert all sample into N gas.
	$\alpha 2,e_{=1.007}$	$^{14}NH3_{(aq)} + 15NH3_{(aq)} \leftrightarrows {}^{15}NH3_{(g)} + {}^{14}NH3_{(g)}$		
Kirshenbaum *et al.* (1947)	αobs=1.008 - 1.023	$^{14}NH4^{+}_{(aq)} + 15NH3_{(g)} \leftrightarrows {}^{15}NH4^{+}_{(aq)} + {}^{14}NH3_{(g)}$	α (the fractionation factor) by mass spectrometry. The isotopic ratio of the sample was obtained by measuring galvanometer deflections caused by the $(^{14N14N})^{+}$ and $(^{14N15N})^{+}$ ion beams.	The fractionation factor for the exchange reaction between ammonia and ammonium nitrate was determined as a function of the dissolved ammonia content. Constant external temperature 25ºC. Equilibration aparatus with mercury displacement device (closed system), samples collected from vapor phase, variable pH (not reported) by NaOH additions. Two nitrogen gas samples per equilibration experiment: one of the vapor phase and one of the liquid phase. Samples oxidated into N with copper oxide.
Farquhar *et al.* (1983)	$\alpha 2,e_{=1.005}$	$NH3_{(aq)} \leftrightarrows NH3_{(g)}$	Compared squared root of the reduced mass of ^{15NH3}/air with ^{14NH3}/air	Calculated the binary diffusivity (ammonia-air bidirectional movement in closed system) ^{15N} enriched systems to measure ammonia volatilization from plant leaves (pH not reported).
	α2,k=1.018	$NH3_{(aq)} \rightarrow NH3_{(g)}$		

Table 3.2 (*cont*) Ammonia isotope fractionation factors (α) experimentally obtained and estimated.

Reference	***Isotope fractionation factor***	***Reaction***	***What was measured of these variables***	***Comments***
Högberg (1997)	α_{eq}=0.980 - 0.983 α_{kin}=1.029	$NH_4^+{}_{(aq)} \leftrightarrows NH_{3(aq)}$ $NH_{3\,(aq)} \leftrightarrows NH_{3\,(g)}$	-	Data collected from various sources
Robinson (2001)	α_{total}=1.040 - 1.060	Ammonia volatilization from soil	-	Data collected from various sources
Li et al (2012)	α_{eq}=0.961-0.951	$NH_4^+{}_{(aq)} \rightarrow NH_{3(aq)}$	$\delta^{15}N_{NH_4^+}$ and NH_4^+ mass fraction of the ammonium remnant in beakers at three temperatures (23, 50 and 70°C. Treatments: static, agitated (stir bar), H_2 bubbling.	No differences in the isotopic fractionation among treatments at 23 °C. Lower Isotopic fractionation at higher temperatures. Assumed that the isotope discrimination between NH_4^+ and NH_{3aq} occurred when ammonium dissociated upon NaOH addition. α_{eq} modeled based on batch equilibrium model.
This research	$\alpha_{obs\ 8.5}$=1.019 $\alpha_{obs\ 9.2}$=1.030 α_{eq}=0.964 α_{kin}=1.050	$NH_{3\,(g)} \rightarrow$ TAN $NH_4^+{}_{(aq)} \leftrightarrows NH_{3\,(aq)}$ $NH_{3\,(aq)} \rightarrow NH_{3\,(g)}$	δ^{15}N by continuous flow stable isotope ratio mass spectrometer. Fractionation factor between TAN and ammonia gas experimentally obtained, and analytical solution proposed for the equilibrium and the kinetic fractionation factors.	Experimental, 22-25º, ammonia was removed by a stream of continuous air (10ml/min). pH=9.2 (buffered solution) and pH=8.5 (Grand River water).

This equation requires that initial and final nitrogen isotopic composition of the solution are known and the product [$10^3 \times \ln_{\alpha NH4+-NH3(aq)}$] is the fractionation factor from the slope of the fitted line. They found that the isotopic fractionation factor α_{eq} for the reaction $NH_4^+ \leftrightarrows NH_{3(aq)}$ was 1.040 at 23ºC. Their estimate is in close agreement with the α_{eq}=0.964 (defined as $NH_4^+/NH_{3(aq)}$) calculated in this experiment; Thode *et al*. (1945) and Högberg (1997) reported α_{eq}=1.027 and 1.031 respectively. It is important to have in mind that temperature affects the solubility of $NH_{3(aq)}$; thus modifying the equilibrium and kinetic alpha values.

Associated error

Unexpected changes in the air flow, handling of serum bottles and possible stoppers leakage were sources of experimental error, thus replicates that were evidently out of the trend were neither included for the estimation of rate constants, nor for the nitrogen isotope delta model. Despite the fact that no statistical differences were found between the observed and the expected isotope mass balance, the variability observed in the mass recovery suggested that the handling and preparation of samples introduced error, in addition to the analytical error. Thus, the standard error of estimates for the model ($s_{e\ pH8.5}$ ±0.0024 and $s_{e\ pH9.2}$ ±0.0025) represents the precision of the calculated α.

As described by Robinson (2001), maximum recovery (>99%) is necessary to decrease the absolute error present in processes with large fractionation factors, such as ammonia volatilization. For the experiment at pH 8.5 with Grand River water, the chemical complexity of the water is high (low mixture purity, following Robinson 2001). The 600 hours duration of the experiment was adequate to evaluate ammonia volatilization from aqueous solutions under controlled conditions with high initial ammonium concentrations (>20 mg N-TAN/L). Improvement in the analytical methods for $\delta^{15}NNH_4^+$ values in samples with very low concentration would be desirable, especially for samples from low concentration environments.

CONCLUSION

The experiments presented here determined the isotopic fractionation factors observed during the volatilization of ammonia in an open system. The overall isotopic fractionation factor α_{obs} from TAN to $NH_{3(g)}$ was calculated as 1.019 (±0.0024) at pH 8.5 and 1.030 (±0.0025) at pH

9.2. With these observed isotopic fractionation factors at two pH values, an analytical solution was developed for estimating the equilibrium isotopic fractionation factor (α_{eq}) and the kinetic isotopic fractionation factor (α_{kin}) . These isotopic fractionation factors were estimated as α_{eq}=0.964 (±0.001) and α_{kin}= 1.050 (±0.003). α_{eq} is in good agreement with previously published results, whereas α_{kin} is higher than previous reports.

CHAPTER 4 - WASTEWATER EFFLUENT PLUMES IN THE CENTRAL GRAND RIVER: AN ASSESSMENT OF THE DISSOLVED INORGANIC NITROGEN CYCLING BEFORE AND AFTER UPGRADES AT THE KITCHENER WASTEWATER TREATMENT PLANT

INTRODUCTION

The quality of surface waters is a growing concern around the world and Canada inland waters are under pressure from a wide range of sources, such as agricultural runoff and wastewater. Wastewater treatment in urban areas is particularly challenging; as the population increases, so does the volume of water requiring treatment and the need to accommodate the treated effluent in surface water bodies. Nutrient inputs from wastewater treatment plants (WTPs) to aquatic ecosystems have to meet certain quality criteria for several reasons, including but not necessarily in order of importance: adverse effects on humans and livestock, aesthetic impairment, interference with anthropogenic uses, negative impacts of aquatic life and excessive nutrient inputs (Doods and Welch 2000). However, treated wastewater is typically high in nutrients. Addition of nutrient-rich wastewater effluent into receiving water bodies have raised public health and environmental concerns, one of the most conspicuous being the increase in primary producers, such as algae and submerged aquatic plants, leading to changes in energy dynamics, habitat and species loss, changes in food web structure, with an overall negative impact the whole ecosystem and its resources and services (Chambers et al. 1997).

A comprehensive review of municipal wastewater treatment plants by Chambers et al (1997) reported that in Canada by the early 1990's, there were 2800 municipal WTP's serving 80% of the population, resulting in approximately 300 litres per capita per day of effluents discharged into surface waters. The Grand River and its tributaries receive effluent from 30 WTPs, in addition to agricultural runoff. Within the Central Grand River, The Region of Waterloo (the largest urban area of the watershed) is facing a remarkable increase in population (approximately 40% in 30 years) that will require an increase in capacity of its WTPs. The Region of Waterloo Engineering and Wastewater Programs group oversees the operation and maintenance of the 13 treatment plants and six pumping stations, including the cities of Waterloo, Kitchener and Cambridge and the townships of North Dumfries, Wellesley, Wilmot

and Woolwich. The effluent from this urban settlement represents a daily total of 200 million litres of wastewater discharged into the Grand River or its tributaries (Region of Waterloo, 2013a); which represents approximately 25% of the observed Grand River discharge during low flow conditions (10 m^3/s at the sampling location downstream of the Kitchener wastewater treatment plant). Considering the estimated population increases, the total treatment capacity required will increase to 282 million litres per day (MLD) by 2031 and 319 MLD for 2041 (Region of Waterloo 2007). Such increases in treatment capacity will be achieved by upgrading the existing WTPs and construction of new WTPs. However, as a result of the WTP's upgrades, concerns have been raised about the downstream river ecosystem health and compromised water quality for municipal water supplies (*e.g.* Brantford, Ontario). The primary water quality concern was the release of ammonium (NH_4^+) from the Kitchener and Waterloo WTPs, which had a large biological oxygen demand downstream, thus leading to hypoxic sections of the river.

The Kitchener wastewater treatment plant (henceforth referred as KTP) underwent a series of upgrades in order to improve the quality of the discharged treated water. The KTP was a conventional secondary treatment plant with activated sludge process with chemical phosphorus removal and sodium hypochlorite disinfection (anaerobic sludge digestion). Due to the fact that the KTP effluent discharged to the Grand River was characterized by high ammonium concentration (NH_4^+), it was important to know the magnitude of the impact produced by the plume created by the KTP effluent in the Grand River. There is not clear information about ammonia or ammonium limit for drinking water in Canada, and no maximum limit for ammonium has been established (CEPA 1999). The limit in ammonia concentration in surface waters has been established by the Canadian Council of Ministers of the Environment that set a Critical Toxicity Value (CTV) of 0.019 mgN-NH_3/L (CCME 2010). The U.S. Environmental Protection Agency (USEPA 2013) established freshwater ammonia water quality criteria for aquatic life normalized to site specific temperature and pH. For example, the chronic criterion magnitude at pH 8 and 20°C is 0.78 mg TAN/L (0.036 mgN-NH_3/L). The nitrate limit for drinking water is 10 mgN-NO_3^-/L (Health Canada 2013)

The schedule for the KTP upgrades was a multistep strategy to increase wastewater treatment capacity and improve quality of the effluent. A sludge dewatering facility was completed early in 2012, UV disinfection and effluent pumping station was completed by 2013, with a projected new secondary treatment plant is to be completed by 2018, where full nitrification is expected (Region of Waterloo 2012b). Summarizing, better aeration led to almost complete ammonium oxidation within the plant by 2013; thus, nitrate was expected to be the dominant nitrogen species entering the Grand River. Thus oxygen depletion in the river would no longer be a concern. Dilution, denitrification and nitrate uptake are expected to regulate in-river nitrate concentration.

The most important change in the quality of the effluent of the KTP due to the upgrades is reduced ammonium discharge. As a result of this change, the nitrogen isotopic composition (*i.e.* $^{15}N/^{14}N$ relative to atmospheric N, or $\delta^{15}N$) of ammonium and nitrate is expected to change. The ammonium and nitrate isotopic composition of a water sample collected inside a treatment plant or in the receiving water body will reflect the physical, chemical and/or biological processes that took place inside the KTP and along the Grand river course as it flows downstream. The processes that change the nitrogen isotopic composition in rivers of nitrogenous species include: nitrification (ammonium oxidation, 15 to 35 ‰; Hogberg 1997, Evans 2007), denitrification (as high as 30 ‰; Sebilo *et al*. 2002), ammonia volatilization (40 to 60 ‰; Robinson 2001), ammonium and nitrate assimilation (≈9 ‰ and up to 19 ‰ respectively; Robinson 2001), groundwater discharge (by mixing waters with different isotopic ratios), among others. These processes yield different relative abundance of ^{15}N; thus, imparting well known differences between the isotopic compositions of the processes involved in chemical and biological transformations.

The objective of this chapter is to characterize and describe the evolution of the plume of nutrients produced by the KTP effluent before and after upgrades during the summer in a section of the Grand River comprising 5700 m downstream of the effluent. Data were collected during summer, low flow conditions when ammonium is high and oxygen is low in the central Grand River (Jamieson et al. 2013), which results in environmental stress, particularly at night. Physicochemical and chemical parameters, together with the ammonium and nitrate isotope

delta were measured, in order to quantify the changes in concentrations and the length of the plume of ammonium created by KTP effluent over four years, representing before, during and after the KTP upgrades. This approach enabled the evaluation of the changes in the water quality of the Grand River as a result of the changes in the KTP effluent, assessing the length of the effluent plume and quantify the changes in ammonium and nitrate concentrations measured in-river as a function of distance and time, emphasizing the relevance of summer as critical season due to low flow, high temperature conditions.

MATERIALS AND METHODS

Study Area

Sampling locations are shown in Figure 4.1 and described in Table 4.1. Five locations downstream the KTP were sampled over a 24 hour period (overnight to the next day), during summer, low-flow conditions, from 2010 to 2013, which represent *before* and *after* upgrades conditions. The sampling location referred to as *upstream* represents the background nitrogen relative to the urban area (21 km upstream of the KTP effluent discharge).

Sampling methods and water analyses

Summer, low-flow conditions were chosen to perform this study, because they represent critical conditions when water quality objectives are most challenging to meet due to low dilution (low base flow discharge), low dissolved oxygen and maximum biological activity (*i.e.* high respiration and high temperature). Five samples were collected per 24 hour period: evening (19:00-20:30), night (21:00 to 22:30), midnight (24:00 to 1:30), pre-dawn (2:30 to 4:00) and afternoon (13:00 to 14:30; Table 4.2). The total distance sampled comprised 5.7 km downstream from the KTP effluent. One upstream location (21 km upstream of the KTP discharge) and the raw effluent from the KTP were also collected. Samples representing the KTP effluent were collected after the de-chlorination pond (2010 to 2012) and at the UV treatment pond (in 2013), prior to being discharged into the river. The sample representing the location at 0 m downstream is the estimated ammonium or nitrate concentration in the river due to instantaneous volumetric mixing of effluent to the river discharge (unless otherwise stated).

Electrical conductivity was measured along cross-sectional surveys at all sampling locations, and the plume was identified as the area of highest EC. Buoys were used to mark sampling locations. Given that the effluent enters the river through a diffuser on the riverbed, it is not possible to collect samples directly at the discharge point.

Table 4.1 - Sampling locations coordinates and distance from Kitchener wastewater treatment plant. Coordinates provided in degrees (*DegDec*). The location BR (Bridgeport) is out of the map (21 km upstream)

Code	Location	Distance from KTP (m)	Latitude	Longitude
BR	Bridgeport	-21000	43.4819	-80.4816
KTP	Kitchener WTP	-	43.401	-80.4322
PT	Pioneer Tower	520	43.938	-80.4155
OMR	Old Mill Rd.	880	43.3947	-80.4149
GC	Doon Valley Golf Club	2600	43.396	-80.3953
FL	Floods (Edgehill St.)	3500	43.4022	-80.3923
BL	Blair	5700	43.3861	-80.3859

Conductivity and pH were measured *in situ* (Hach Multiprobe HQ40d). Water for chemical analyses was collected in HDPE containers to be sub-sampled and filtered (0.45µm). Ammonium (NH_4^+) and nitrate (NO_2^-) were analyzed by colorimetric methods (blue indophenol and with sulphanilamide + azo dye respectively) using a Beckman UV spectrophotometer and Smartchem 200 Autoanalyzer (precision 5%, detection limit 0.05 mg N/L). Ammonium samples were acidified prior to analysis, thus TAN and NH_4^+ represent NH_3+NH_4^+ henceforth Nitrate (NO_3^-) and chloride (Cl^-) were analysed with an Ion Chromatograph (Dionex ICS-90; precision 0.07 mg N/L, detection limit 0.05 mg N/L). Total Nitrogen was analysed by acid combustion in Apollo 9000 Combustion TOC/TN Analyzer (Teledyne Tekmar) and Shimadzu TOC-L Total Organic Carbon Analyzer with TNM-L Total Nitrogen Measuring Unit (precision 0.3 mg C/N L^{-1}). Dissolved oxygen was analysed by Winkler titration (precision 0.2 mgO_2 L^{-1}).

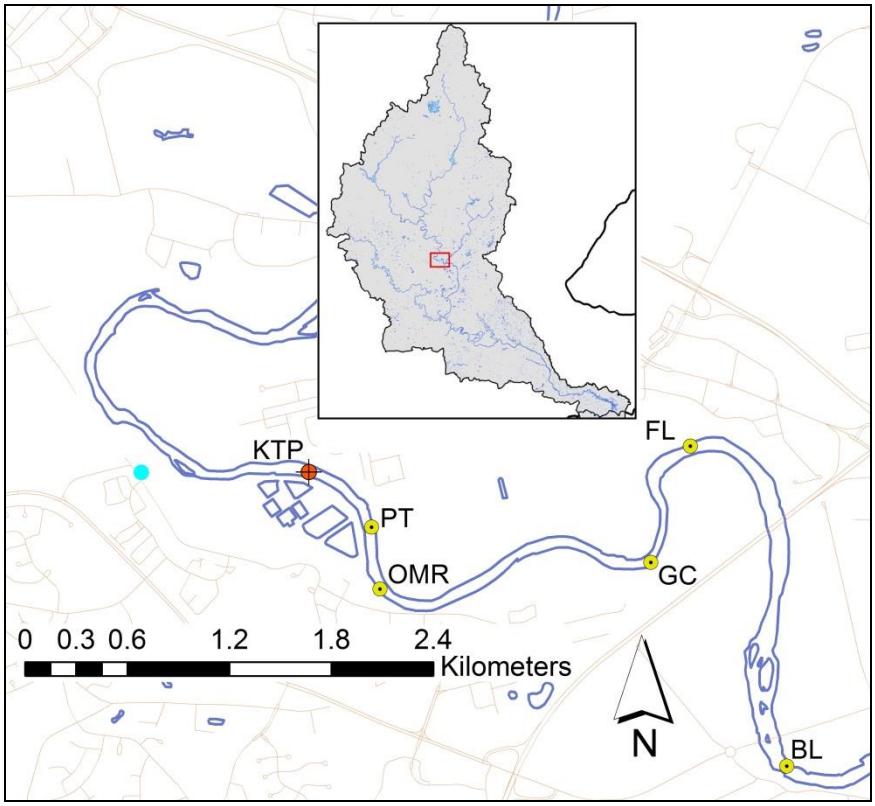


Figure 4.1 - Sampling locations downstream of the Kitchener Wastewater Treatment Plant (KTP). Rectangle represents the section sampled within the Central section of the Grand River (City of Kitchener). The upstream sampling location is 21 km upstream of the KTP (out of the map) Effluent discharge is represented by KTP crossed circle. For details about location, refer to Table 4.1

Table 4.2 - Plume sampling events.

Year	Date	Time of the day	Sampling events
2010 *(Before)*	July 7-8th	19:00 to 14:00	5
2011 *(Before)*	July 13-14th	19:00 to 14:00	5
2012 *(Before)*	July 18-19th	19:00 to 14:00	5
2013 *(After)*	August 29th	9:00 to 12:00	1

All data were chloride-corrected [Cl/Cl_0] to account for dilution. Chloride correction was done by setting the chloride value at the location closest to the KTP effluent (520 m, Pioneer tower) as the initial chloride value at each sampling time; then the chloride concentration at the next sampling location was used to calculate what could have been the NH_4^+ or NO_3^- concentration if no changes in chloride have occurred. Chloride was slightly variable as the plume of nutrients traveled downstream (Figure 4.2), meaning that not all samples were always collected in the ideal centre of the plume.

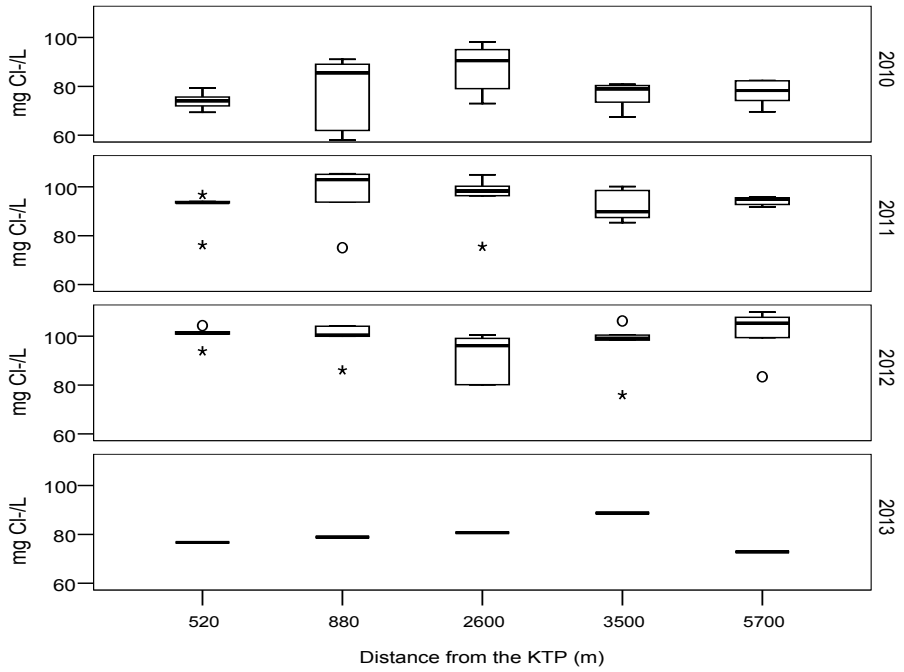


Figure 4.2 - Chloride concentration (mg Cl^-/L) measured in the Central Grand River during the *plume sampling* events, summer low flow condition between 2010 and 2013. 2010 to 2012 was sampled at different times of the day, 2013 was sampled once during day time. Boxplots show the median (*black line*), interquartile range (*boxes*), outliers (*circles*), and extreme cases of individual variables (*asterisks*).

Given the uncertainty of the boundaries of the effluent plume, no areal or flow-weighted results are presented here. The rates of NH_4^+ decrease and/or NO_3^- increase were calculated per metre of river length. A travel time estimation was provided by GRCA or derived from flow and channel morphology (average width and depth at sampling point) to calculate rates per time. Reporting concentrations or fluxes were used where appropriate.

Estimates of ammonia volatilization

The free ammonia (NH_3) is the fraction of the total ammonia nitrogen concentration at given pH and temperature conditions, calculated as $f = 1/(10^{pKa-pH} + 1)$; where the acid dissociation constant pKa=0.09018 + (2729.92/T). Estimates of ammonia flux to the atmosphere in the Grand River were completed by assessing the mass transfer corrected for Schmidt number (Sc_{NH3}), using the oxygen mass transfer coefficient modelled in the Central Grand River during summer, k_{O2}≈0.2 m/h (Venkiteswaran et al. *unpublished*). Correction for ammonia was done as follows:

$$k_{NH3} = \frac{k_{O2}}{\left(Sc_{O2} / Sc_{NH3}\right)^{-0.5}} = 0.19\, m/h$$

The flow-adjusted rate of volatilization was calculated using summer, low flow velocities measured by the Grand River Conservation Authority (GRCA) from 0.3 to 0.4 m/s between the KTP discharge location and the sampling station five kilometres downstream the KTP outflow (Blair).

Nitrogen isotopes analysis

Nitrate and ammonium nitrogen isotope delta were analysed to gain a better understanding of the processes occurring in the raw effluent and in the plume of nutrients created in the Grand River. $\delta^{15}N_{NO_3^-}$ was analysed by chemical denitrification (conversion of NO_3^- to N_2O) as described by McIlvin and Altabet (2005). Samples for $\delta^{15}N_{NH_4^+}$ were acidified in the field (to pH=4) and processed in the laboratory by an alkaline diffusion method following Spoelstra *et al*. (2006); an alkaline solution (4 M KCl) was prepared so that a total volume of 20 mL (standard or sample) is placed in a 50 mL glass Teflon-lined jar, with an approximate mass of 15 μg of N per sample. Diffusion traps were prepared by sealing acidified (10 μL 0.2 M H_2SO_4) quartz filter disks (Whatman QMA filters, baked at 550 ºC) in a section of polytetrafluoroethylene (PTFE) tape ('T-Tape'), in order to allow complete diffusion of the NH_3 onto the acidified disks. Each unit was adjusted to alkaline conditions (0.2 M NaOH) and buffered (pH ~9.3) with 2 mL of tetraborate buffer (0.1 M $Na_2B_4O_7$ and 0.075 M NaOH). The jars were shaken continuously (80 rpm). After 10 days the PTFE traps were removed and the filter disks retrieved and placed in 4.5 mL glass vials, frozen and then freeze dried. All isotopic analyses for nitrate and ammonia were determined with a Delta Plus, Continuous Flow Stable Isotope Ratio Mass Spectrometer (Thermo Finnigan/Bremen-Germany) coupled to a Carlo Erba Elemental Analyzer (CHNS-O EA1108 - Italy). Stable isotope ratios are expressed as delta (δ) values and are expressed as per mil (‰). Stable isotope analyses were performed at the Environmental Isotope Laboratory of the University of Waterloo with an analytical precision of ±0.3‰ for ^{15}N. Standards were prepared from IAEA 311 ammonium sulphate salts, characterized by the EIL – University of Waterloo using IAEA-N-1 (+0.43‰), IAEA-N-2 (+20.41‰) and IAES 305B (+39.8‰).

RESULTS AND DISCUSSION

Changes in quality of the KTP effluent

Raw effluent in the KTP monitored by the Region of Waterloo in July (as representative of summer, low-flow conditions), had total ammonia nitrogen (TAN=NH_3+NH_4^+) between 21 to 26 mg N/L in 2011, 13 to 21 mg N/L in 2012 before upgrades, and only 0.8 - 5.4 mg N/L in 2013, after the upgrades. Consequently, nitrate concentration in the effluent discharged by the KTP increased from 3.8-7.6 mg N-NO_x^-/L (NO_x^-=NO_2^-+NO_3^-) in 2012 to 21-31 mg N-NO_x^-/L in 2013. The nitrogen discharged from the KTP was lower before upgrades (1681±216 kg N-DIN per day) than after upgrades (1905±236 kg N-DIN per day, Student's *t*=2.66, t=0.01)

Despite the fact that enhanced aeration inside the KTP started in August 2012, high variation in ammonium and nitrate concentrations was observed early in the year 2013 (Region of Waterloo 2014). Figure 4.3 shows the variability in the water quality of the effluent. TAN concentrations decreased from 20 mg N/L in January 2013, to 5mgN/L by April 2013. The reduction in TAN concentrations resulted in increased concentrations of nitrite+nitrate in the effluent (higher than 20 mg N/L consistently observed after April 2013. This change in effluent quality was the result of full nitrification completed inside the KTP (Region of Waterloo 2014).

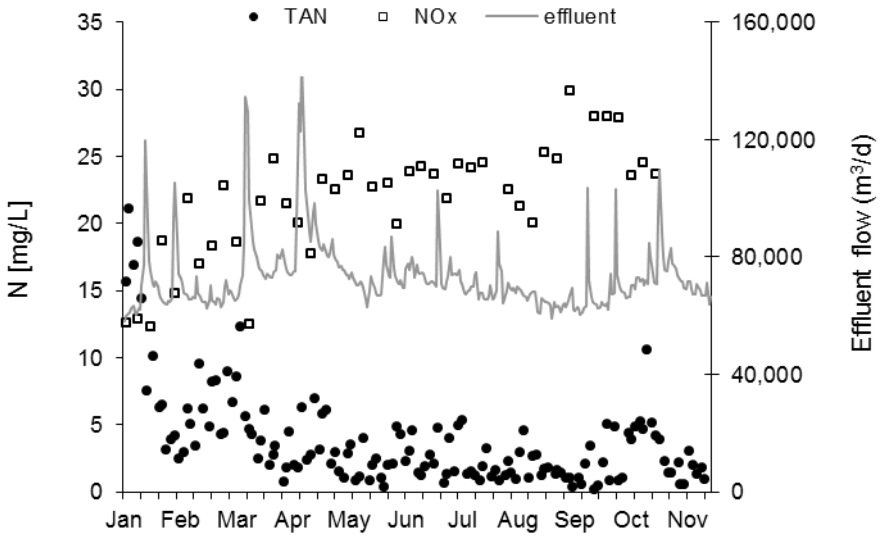


Figure 4.3 - Changes in quality of Kitchener wastewater treatment plant effluent during upgrades completed in 2013 (Data provided by the Region of Waterloo). TAN: Total Ammonium Nitrogen (ammonia+ammonium); NO_x: nitrite+nitrate.

Setting the stage: physicochemical conditions in the Central Grand River

The ecosystem health of the Central Grand River is tightly linked to the operation of sewage plants: the water quality of the river is primarily impacted by wastewater treatment plant effluent quantity and quality. Additionally, seasonal and daily variations in water chemistry are relevant when assessing the impact of a wastewater treatment plant. Nimick *et al*. (2011) reviewed some of the major biogeochemical changes observed in stream ecosystems over a 24 hour period. These changes are both physical and chemical, chiefly driven by temperature, dissolved oxygen produced during photosynthesis, and pH changes due to changes in uptake and production of CO_2. During summer sampling, water temperature at the study sites varied around 2 ºC over the 24 hour period. Locations closer to the KTP discharge oscillated less, possibly because the continuous contribution from the effluent with a temperature higher than the river water. Occasional sampling during fall and spring revealed that the KTP effluent had higher temperatures that the receiving water and that the increase in temperature (compared to upstream locations) was greater than 2ºC, which made temperature an additional mean of identifying and tracing plumes of nutrients created by wastewater treatment plant discharge. This method would work best in rivers with low or zero groundwater input.

pH varied less than 1 unit (data not shown) over the day, and its effect upon free ammonia (and its subsequent volatilization) could be important given its effect upon the free ammonia available. Even though proton-exchange reactions (ammonium oxidation) took place along the plume of nutrients studied, the Grand River is a well buffered system due to the local geology (dominantly limestones) and pH is commonly above pH=7. The dilution achieved in the river once the effluent is discharged (see *Plume delineation* below) can also reduce impacts from the KTP effluent. Wastewater effluent to river discharge ratios (Q_{WTP}/Q_{river}) from 0.25 to 1 have been studied by Gammons *et al*. (2011) and Ribot *et al*. (2012) in small streams, where all water flowing in those systems originates in a WTP. Such conditions created evident impacts on the receiving waters due to the large volume of the effluent, high nitrification and denitrification rates and resulted in very high $\delta^{15}N_{NH_4^+}$ values.

Changes in dissolved oxygen, dissolved organic carbon and phosphorus before and after KTP upgrades

Before the upgrades were completed, high ammonium was discharged from the KTP because the former operation consisted of superficial aeration, which resulted in low dissolved oxygen in the water column of the oxidation ponds and reduced NH_4^+ oxidation inside the KTP. The effluent leaving the KTP was typically only about 50% saturated in dissolved oxygen (~ 4mg O_2/L, Rosamond 2013).

The daytime dissolved oxygen in the Grand River during the summer before upgrades (2010 to 2012) was commonly between 6 and 9 mg O_2/L upstream of urban area. The low dissolved oxygen in the effluent discharged by the KTP impacted the dissolved oxygen in the Grand River, observed as a decrease in the oxygen saturation in the water downstream of the KTP due to the high chemical oxygen demand due to the high ammonium discharge. The pattern of dissolved oxygen recovery downstream of the KTP was variable among years and almost recovered the oxygen saturation observed upstream of the KTP effluent (Figure 4.4, left panel).

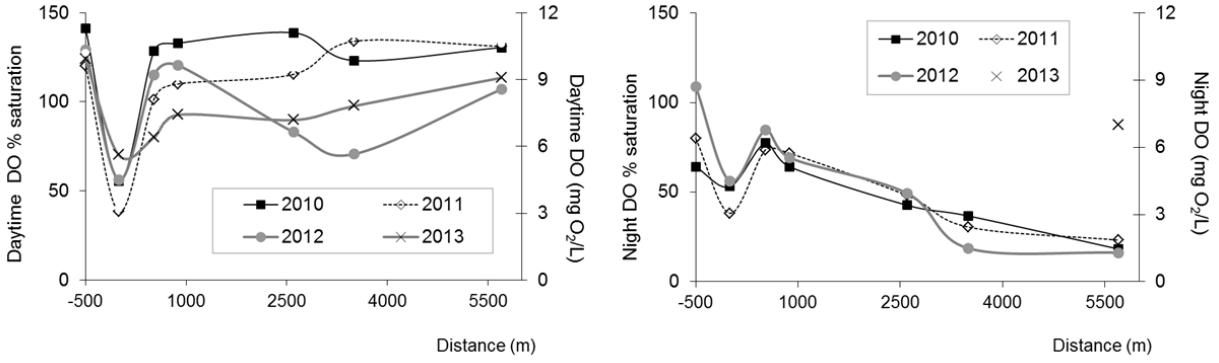


Figure 4.4 - Oxygen saturation (%) and dissolved oxygen (D.O) in milligrams per litre. D.O. concentration and saturation at 0 metres represents samples collected inside the Kitchener wastewater treatment plant, samples for 2013 represents *after* KTP upgrades conditions. *Left panel*: samples collected after solar noon (1:00-3:30 PM). *Right panel*: samples collected during night time (2:00-4:00 AM). Samples collected inside the plume of nutrients below the KTP. 2013 nighttime D.O concentration (7 mg O_2/L) obtained from the GRCA sampling station "Blair". % saturation calculated with USGS DOTABLES (http://water.usgs.gov/software/DOTABLES/).Calculations valid for water temperature 0-40 °C, barometric pressure 380-836 mm Hg, specific conductance 0 to 59118 µS/cm².

The dissolved oxygen concentration at night (2:00 to 4:00 AM) before upgrades dropped dramatically in the 5700 m reach of this study (Figure 4.4, right panel). The high ammonium concentrations discharged from the KTP consumed overnight most of the dissolved oxygen in the water column, thus it was observed hypoxic conditions in the central reach of the Grand River downstream the KTP at night, when photosynthetic oxygen evolution is not active (Figure 4.4, right panel). The impact of the KTP effluent was more important during the night than during the day because the photosynthetic oxygen evolution did not supply the oxygen used during nitrification. The KTP upgrades included submerged bubblers in the oxidation tanks, resulting in more efficient ammonia oxidation. Night time sampling was not done after upgrades (in 2013); however, the lowest dissolved oxygen concentration measured by the GRCA at the sampling location Blair (5700 m downstream of the KTP) was approximately 7 mg O_2/L (81% D.O saturation) during the night of the sampling event (August 29th 2013). The reduction in ammonium discharged from the KTP and the higher-than-before dissolved oxygen measured inside the KTP (6.1 mg O_2/L) prevented the summer night-time hypoxic conditions downstream to the KTP effluent.

Stoichiometrically, one mol of ammonium requires two moles of oxygen to be completely converted into one mol of nitrate (Wetzel 2001), which represents chemical oxygen demand (COD) of approximately 4.5g COD per gram nitrogen as ammonia. Before upgrades (July 2011), COD was estimated to be from 123 to 147 g $CODs^{-1}$; which was reduced to 5.82 g $CODs^{-1}$ in 2013 after upgrades; representing a decrease in 96% in the COD. Additionally, there is oxygen demand associated with organic carbon molecules in the effluent and changes to DO production and consumption due to increased biological productivity downstream of the KTP.

The dissolved organic carbon (DOC) and phosphorus (TP and SRP) concentrations in effluent also changed after the KTP upgrades, considered to be caused by dilution effects and variable effluent volume discharged from the KTP. Carbon and phosphorus transformation will not be considered here; however, an increase in phosphorus from upstream conditions was observed, whereas DOC was not significantly different from upstream conditions within the same year. A very comprehensive review on phosphorus trend in the Grand River, spanning decades, has been prepared by Hood (2012).

Effluent DOC measured in July and August (2010-2013) ranged from 6.7 to 12 mgC/L, with a very high value of 22 mg C/L in July 2011. Hutchins (2012) compared longitudinal and seasonal trends in DOC along the Grand River and found that WTP effluents stimulate primary production; thus, generating autochthonous DOC in the river. High DOC, together with phosphorus increase as a result of human impacts caused large seasonal cycles and potential threats to drinking water quality due to the generation of disinfection by-products (Hutchins 2012).

Dissolved gases were not thoroughly examined in this study, although processes discussed here (nitrification, denitrification, respiration) produce greenhouse gases (N_2O and CO_2). Greenhouse gases are typically elevated in the central Grand relative to the rest of the river, are above atmospheric saturation (i.e. are a source to the atmosphere) and are highest at night (Jamieson et al. 2013, Rosamond et al. 2011, Venkiteswaran et al. 2013, Venkiteswaran et al, *in review*).

Plume delineation

Cross-sectional surveys completed before all sampling events assisted in locating the centre of the plume of nutrients. Figure 4.5 shows an example of an overnight survey (July 2011) that identifies the wastewater plume. The effluent (with electrical conductivity above 2000 μS/cm) was discharged into the Grand River through a submerged diffuser, and underwent rapid dilution within the first 500 metres. The plume was consistently located close to the west or south bank of the river. However, the centre of the plume was not located in at the same position over the four years of study. Additionally, the river bed changed over four years due to erosion and deposition.

The plume of nutrients was not a confined mass of water; rather it was a pocket of water within the river with distinctive chemical properties that slowly homogenizes downstream due to simultaneous advection and dispersion. It is possible that the plume of nutrients eventually disappeared; however, the chloride concentration as far as 5700 m downstream of the effluent (Figure 4.2) suggests that once the diffuser discharged into the Grand River, the plume is relatively cohesive as it travels downstream.

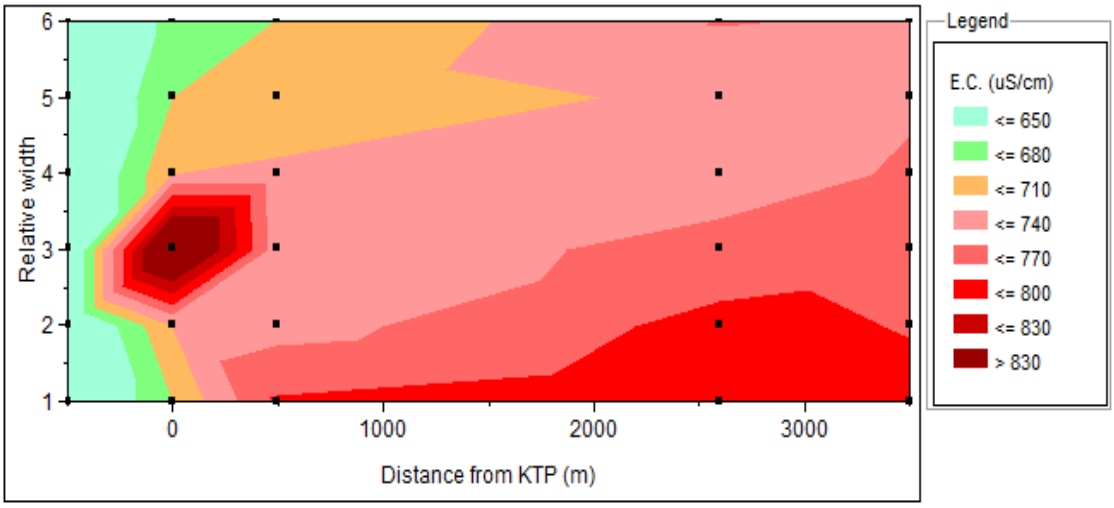


Figure 4.5 - Contour plot representing the plume of nutrients in the Grand River, after receiving discharge form a wastewater treatment plant. Notice the relatively well-confined mass of water that represented the centre of the plume. Data representing summer, low-flow conditions (July 2011). The *y* axis is relative and the river does not necessarily have a consistent width.

There are two methods for quantifying dilution: the *wastewater:river water* ratio and the dilution factor estimated from dilution of a conservative tracer such as chloride (Cl/Cl_0). The *wastewater:river water* ratio is meaningful when the receiving water body is small enough to allow quick and complete mixing. Chloride-dilution is used when assessing a plume of nutrients in large rivers, because chloride acts as a conservative tracer that allows estimating incomplete mixing in the water column. The average chloride dilution ratio was 0.15 in 2010, 0.17 in 2011 and 2010, and 0.16 in 2013; and the variability observed (from 0.08 to 0.22) could be attributed to different volumes of effluent discharged into the Grand River between day and night time. Discharge is frequently greater during the morning (10:00 AM to 12:00 PM) and lower at dawn (2:00 to 4:00 AM, Figure 4.6). Thus, base flow discharge of the Grand River and the variable effluent from the KTP regulate the dilution ratio, which affects the ammonium and nitrate concentrations measured in the river.

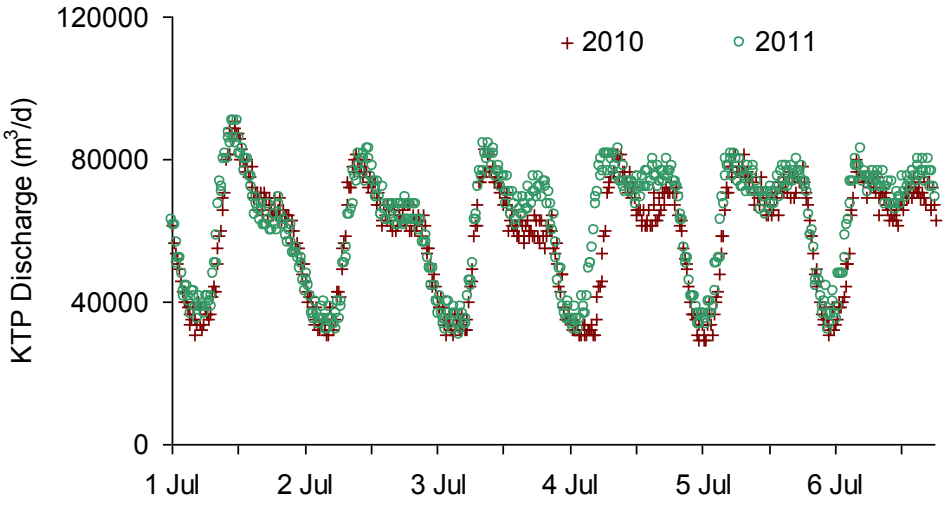


Figure 4.6 - Day/night oscillations in the effluent discharge by the Kitchener Wastewater treatment plant (m^3/d) every 15 minutes. Data obtained from Region of Waterloo. Notice the two daily peak behaviour of the effluent.

Estimation of the plume length

As opposed to nitrate, a maximum limit for ammonium in drinking water does not exist; thus, the Critical Toxicity Value (CTV) of 0.02 mgN-NH_3/L established by the Canadian Council of Ministers of the Environment (CCME 2010) was used as a guideline to determine the length of the plume of nutrients developed downstream of the KTP. This value was surpassed in several summer sampling events before the KTP upgrades, which means that dissolved ammonia concentrations (NH_3) were likely an ecological risk at various locations close to the KTP (Figure 4.7). Some sampling events before the upgrades had estimated free ammonia almost ten-times different between day and night, given the differences in NH_4^+ concentrations (around 0.5 mgN-NH_4^+ /L) and that the pH was 0.7 units lower during the night than during the day.

The KTP effluent created a *high ammonium* zone for at least 5000 metres downstream of the KTP in the Central Grand River. The length of a nutrients or contaminants plume depends on the river discharge, the sewage volume disposed into the receiving river and the water temperature. For example, in a relatively narrow and shallow river in Finland (30 m width, 3 m depth; discharge ≈ 7 m^3/s), pharmaceutical products were detected as far as 5 km downstream of a WTP in a cold month (March), but undetected in May and August (Vieno et al 2005).

Rivers with higher discharge (e.g. the Bow River, summer discharge ≈150 m^3/s) generate larger plumes (estimated in 46 km length for the Bow River downstream Calgary, Chung 2013). It is important to stress that dissolved ammonia CTV (NH_{3diss}) could only be estimated when water quality monitoring reports include pH and temperature; thus, it is important to maintain continuous monitoring of those parameters in order to properly assess the ammonia toxicity threshold.

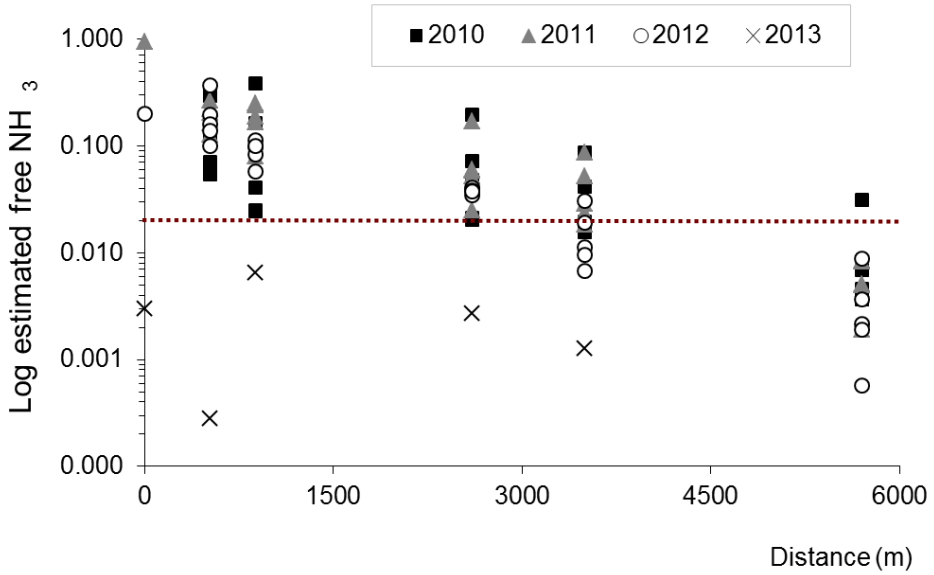


Figure 4.7 - Length of the plume of ammonia formed downstream of the Kitchener wastewater Treatment Plant, calculated from pH and TAN concentration. The dashed line represents the guideline of ammonia for the protection of aquatic life (CCME 2010). Total ammonia nitrogen was below detection limit at the location 5700 m in 2013.

In the summer of 2013 (after upgrades), ammonia plumes exceeding the CTV were no longer observed. The KTP effluent concentration after upgrades (August 2013) was 23 mgN-NO_3^-/L; which was diluted as soon as it was discharged into the Grand River. In order to meet ecological requirements and overcome critical periods due to high nitrate concentrations, the GRCA has established 3 mg N-NO_3^-/L as the target concentration for the watershed, in agreement with the nitrate guideline for Canada (GRCA 2013).

Observed ammonium decrease downstream of the KTP

Due to the fact that the samples were only collected in an unconfined plume of nutrients without a well-defined surface area, estimation of areal rates of TAN and NO_3^- loss or gain was not possible.

The decrease of ammonium concentration observed during summer, low flow conditions at different times of the day fit a first order decrease $[TAN]=[TAN]_0 \cdot e^{-kd}$, with d=distance in metres (Figure 4.8). Rate constant k between 0.13×10^{-4} and 0.72×10^{-3} m^{-1} were observed before the KTP upgrades. Rate constants were lower at midnight because ammonia oxidation and possibly plant uptake were not as intense as during the day. Volatilization rates were very similar (see *Ammonia volatilization estimates* below). The Table 4.3 describes the observed ammonium decrease and the rate order k for all sampling events.

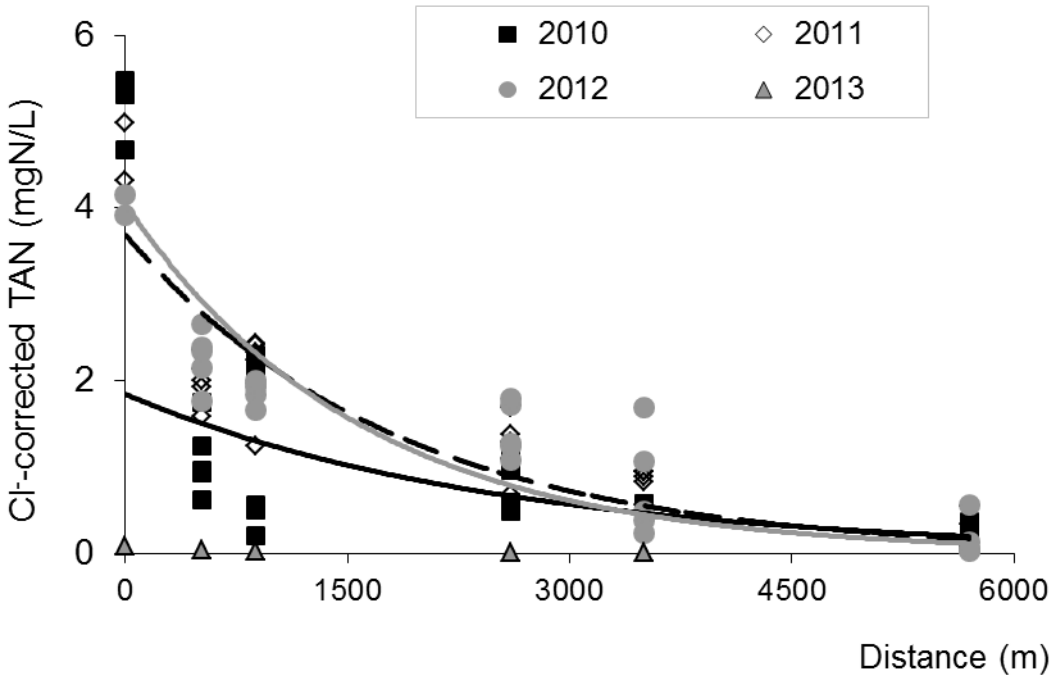


Figure 4.8 - Observed Total Ammonia Nitrogen (chloride-corrected for dilution) with distance within the plume of nutrients developing downstream of the Kitchener wastewater treatment plant. The centre of the plume is the location with the highest electrical conductivity.

There was a very clear trend in high ammonium concentration during the night compared to daytime samples. This is the result of less intense ammonia oxidation during the night, when

photosynthetic oxygen evolution is not active and low dissolved oxygen concentrations in the central Grand River favour the presence of high ammonium concentrations in the water column. A similar trend in day high-night low ammonium concentration (and ammonium concentration decrease with distance) was described by Gammons et al (2010) in a creek receiving WTP effluent.

Table 4.3 - Ammonium decrease rate constant before (2010-2012) and after (2013) Kitchener wastewater treatment plant upgrades. Rate constant *k* in s^{-1}, data adjusted by travel time (provided by GRCA and estimated in field).

Year	Round	Rate constant *(k)*	r^2	Comments
2010	Evening	2.6×10^{-4}	0.21	Base flow (11m^3/s) similar to historical normal. Few locations appeared to be outside the plume (evening and midnight)
	Night	3.8×10^{-4}	0.70	
	Midnight	6.3×10^{-5}	0.34	
	Dawn	2.4×10^{-4}	0.66	
	Noon	5.1×10^{-4}	0.92	
2011	Evening	6.9×10^{-4}	0.88	Base flow (7 m^3/s) below historical normal. Data were better constrained and consistent among sampling times.
	Night	6.2×10^{-4}	0.85	
	Midnight	3.8×10^{-4}	0.94	
	Dawn	1.4×10^{-4}	0.63	
	Noon	8.0×10^{-4}	0.88	
2012	Evening	7.7×10^{-4}	0.98	Base flow (8 m^3/s) below historical normal. Data were consistent among sampling times. Plume located at different location (cross-section) than previous years
	Night	8.4×10^{-4}	0.89	
	Midnight	5.4×10^{-4}	0.87	
	Dawn	1.6×10^{-4}	0.55	
	Noon	7.2×10^{-4}	0.88	
2013	Noon	8.7×10^{-5}	0.95	Base flow (12 m^3/s) above historical normal. Low ammonium discharge ("after" conditions)

During all sampling events, ammonium concentration decreased downstream in the plume, from more than 1 mgN-NH_4^+/L at 520 metres below the KTP, to around 0.2 mgN-NH_4^+/L at 5700 metres downstream the KTP. There were differences between the volume of the effluent discharged by KTP before upgrades (higher in 2012 than the other years, Tukey-Kramer HSD q=2.383, p=0.0001); but no significant difference between years was observed for ammonium concentration before upgrades (ANOVA F=1.596). For this reason, the differences observed between years are related to variable river flow and volume discharge from the KTP.

Ammonium concentration in-river after the KTP upgrades (2013) decreased to less than 0.2 mgN-NH_4^+/L in the sampling location closer to the KTP discharge due to the low ammonium concentration in the KTP effluent after upgrades in addition to the dilution one the effluent was discharged into the Grand River.

Ammonium decrease: travel time-adjusted

Data provided by the Grand River Conservation Authority and field measurements (floating buoys assessment) under different discharge regimes (9 to 14.5 m^3/s) were used to estimate flow velocity in the river. In summer, a flow velocity of 0.3 to 0.4 m/s was calculated during low flow conditions. Total ammonium loss for the reach of the river comprising 5700 meters was calculated per length per time (Table 4.4), representing the net changes in ammonium mass between the two sampling locations separated 5700 m from each other (*i.e.* initial and final conditions).

Table 4.4 - Ammonium decrease rates chloride-corrected for dilution and adjusted by travel time. Flow velocity= 0.3 m/s. Samples collected inside the plume of nutrients developed downstream the Kitchener wastewater treatment plant. Rates calculated for a total distance of 5700 m (*i.e.* initial and final NH_4^+ concentrations).

Year	µgN-NH_4^+ m^{-1}	mgN-NH_4^+ h^{-1}	µgN-NH_4^+ s^{-1}
2010	0.87	2.47	0.69
2011	0.78	0.80	0.22
2012	0.68	0.70	0.20
2013	0.04	0.11	0.03

The decrease in NH_4^+ concentration in samples collected inside the plume of nutrients is the result of nitrification, biological assimilation, volatilization and dispersion of the plume. Ammonium decrease during 2011 and 2012 (years with below-average base flow) was three times lower than those observed in 2010, a year with historical average base flow. This is attributed to reduced dispersion, because low velocity allowed for a higher and more sustained concentration. Additionally, ammonium removal was similar per unit length among years, but they are different per unit time (larger in 2010) confirming that travel time (*i.e.* flow velocity) is an important factor of ammonia removal estimates.

Ammonium removal was greatly variable; however most of the rates were lower than 0.4 µmol NH_4^+ m^{-1} (Figure 4.9). Although sampling locations were marked with buoys and the sampling strategy was consistent among years, variable behaviour observed can be attributed to plume migrations during the day, due to slight variations in flow velocity, water depth or movement of the ideal centre of the plume. Ammonium adsorption to sediment was not estimated in this research given that cobbles and gravel is dominant in this section of the Grand River. Rapid decay of organic matter or in-stream cycling cannot be ruled out, although it is beyond the scope of this research.

Given that this research reports data from a plume of nutrients, areal or volumetric rates were not estimated; however, similar studies reported nitrification rates of 0.02 to 0.09 mmol N l^{-1} h^{-1} in a small stream, where treated wastewaters could account for up to 100% of stream discharge (Gammons et al. 2010) and 3 mgN l^{-1} (≈0.21 mmol N l^{-1}) in the water column in the lower Rhine (Admiraal and Botermans 1989).

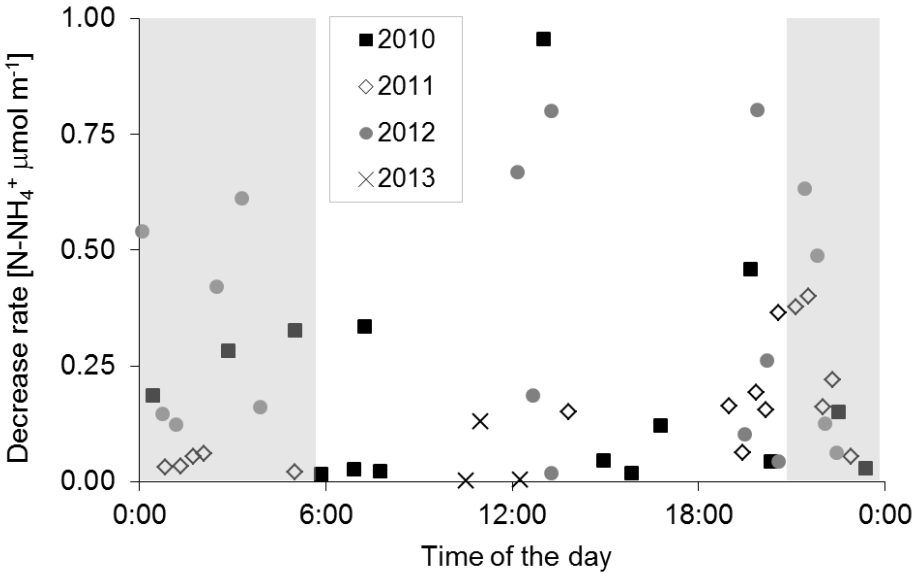


Figure 4.9 - Ammonium decrease rates (µmol N-$NH_4^+$$m^{-1}$) by year within the plume of nutrients below the Kitchener Wastewater treatment plant. Shaded panels represent night hours. Rates calculated with chloride-corrected ammonium concentration adjusted by travel time (flow velocity=0.3 m/s).

Ammonia volatilization estimates downstream of the KTP

The volatilization of ammonia in the Grand River was estimated as the ammonia flux from the water column into the atmosphere using the stagnant film model of gas transfer (Denmead and

Freney 1992). For the Central Grand River, Venkiteswaran et al. (*unpublished)* measured an oxygen mass transfer coefficient (k_{O2}) below 0.2 m/h in the Central Grand River during summer conditions, thus the ammonium mass transfer coefficient can be obtained from the measured oxygen mass transfer coefficient corrected by the Schmidt number. The Schmidt number (*Sc*) is the ratio of the kinetic viscosity to the mass diffusivity of a gas, it decreases as the diffusivity of the gas increase and does not depend much on temperature (Loubet *et al*. 2009)

$$k_{NH3} = \frac{k_{O2}}{\left(Sc_{O2}/Sc_{NH3}\right)^{-0.5}} = 0.19\, m/h$$

With Schmidt number Sc_{O2} = 380 (following Wanninkhof 1992) and Schmidt number Sc_{NH3}=410; therefore Sc_{O2}/Sc_{NH3} = 0.92. Therefore, the calculated ammonia mass transfer coefficient k_{NH3} was 0.19 m/h in the central Grand River for the summer of 2010 to 2012 under low flow conditions and water temperature around 25ºC. With this ammonia transfer coefficient and flow velocity set in 0.3 m/s (1080 m/h), instantaneous flow-weighted fluxes (g N per square meter per hour) for the five locations below the KTP are shown in Figure 4.10 with no distinction among years. Greater flux at the location 880 m from the KTP was probably due to the shallow water column during the sampling events (20 to 30 cm).

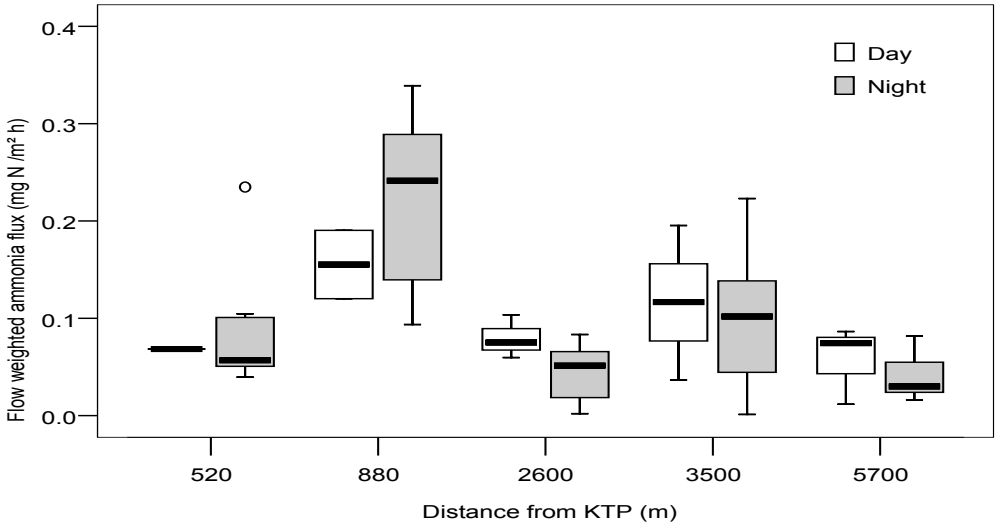


Figure 4.10 - Flow-weighted ammonia flux (in mgN-NH^3/m^2 h) at the five locations downstream of the Kitchener wastewater treatment plant during summer, low flow conditions (2010-2012). Flow velocity = 0.3 m/s. Day time sampling was done between 13:00 and 16:00 hours; night time sampling between

19:00 and 4:00 hours. Boxplots show the median (*black line*), interquartile range (*boxes*) and outliers (*circles*).

The cumulative flux of the reach of the central Grand River from the KTP effluent to the location at 5700 m downstream of the effluent (mgN-NH_3 m^{-2} h^{-1}) is shown in Figure 4.11. Despite the fact that the pH changed between 0.5 and 0.8 pH units (lower during the night) and the water temperature varied around 2°C between the day and the night, the ammonia flux was similar between day and night before upgrades, slightly higher in the day in 2010. The fact that four samples were collected during the night time and only one sample represented the daytime prevents the comparison of the cumulative rates in daytime and its variability (Figure 4.11)

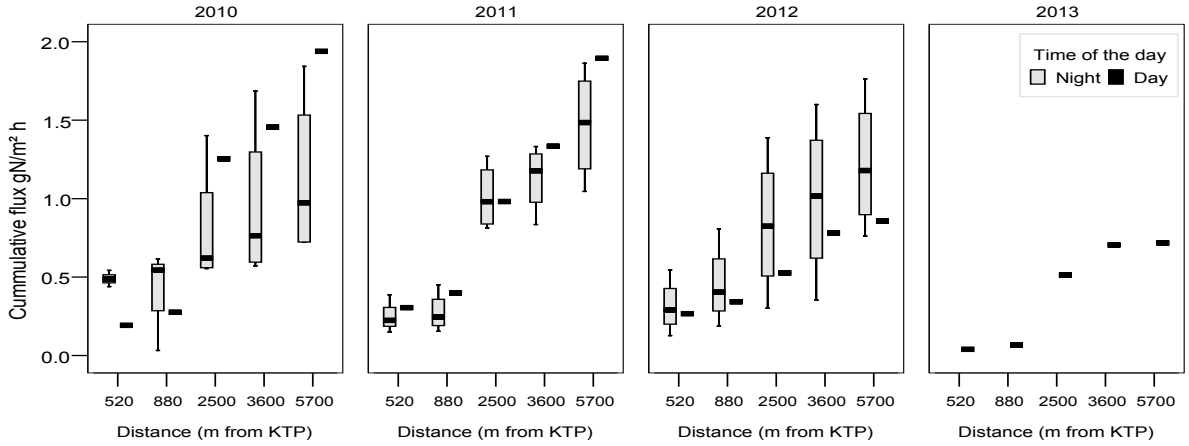


Figure 4.11 - Cumulative flux of ammonia by volatilization (*F*, gN $m^{-2}h^{-1}$) within the plume of nutrients downstream the Kitchener wastewater treatment plant during summer, low flow conditions (2010-2013). Flow velocity = 0.3 m/s. Night time sampling was done between 19:00 and 4:00 hours (n=4), day time sampling was done after solar noon (n=1).

The cumulative loss was lower in the years with flow below-historical average (2011 and 2012) because of the slower river flow. It is important to highlight that the flux estimates expressed in mass per surface area per time have been calculated with water samples collected within the plume of nutrients; thus, representing the section of the Grand River with the highest concentrations of ammonia. Lower fluxes should be expected in the fringes of the plume. Flux calculations were not done for the year 2013 (after upgrades) given that reduced ammonium concentration in the effluent would represented small ammonia flux.

In order to contribute to the data reported by the Provincial water quality monitoring network (PWQMN), ammonia volatilization at the location 5700 m downstream of the KTP (Blair, PWQMN # 16018401202) is presented in Table 4.5. The estimates of N loss due to ammonia volatilization, represents between 50 and 60% of the TAN originated inside the KTP within the first 5.7 km stretch of the river. To my knowledge, there are not previous estimates of ammonia volatilization from a river. Ammonia volatilization in rivers has been simulated in continuous airflow enclosures, thus representing the potential capacities of urban rivers to remove ammonia by volatilization. The estimated total ammonia removal form the simulated aeration experiments was between 18 and 39%, with measured rates as high as 0.01 g N $m^{-2}h^{-1}$ (Liu et al 2013), much lower than the flux estimated and showed in Figure 4.10.

The total mass of ammonia volatilized depended on factors such as pH, temperature, variable discharge from the KTP, river discharge and flow velocity. Ammonia loss might be limited by gas diffusion through ice in winter, when low temperatures and ice cover on the water surfaces reduces the ammonium mass transfer coefficient, thus decreasing ammonia volatilization during winter, allowing to observe high ammonia concentrations during this season. Even though ice may not cover the central Grand River fully due to the continuous supply of warmer water from the KTP, it is a physical barrier to gas transfer between the water and atmosphere (Silvennoinen *et al*. 2008).

Table 4.5 - Ammonia rate loss (mgN-NH_3 $L^{-1}m^{-1}$) downstream of the Kitchener wastewater treatment plant during summer, low flow conditions (2010-2012). Ammonium loss calculated form concentration chloride-corrected for dilution and adjusted by travel time (flow-weighted fluxes); k=0.18m/h. TAN_0=2.2 to 2.65 g TAN/L (estimated concentration due to *effluent:river* dilution). Flow velocity provided by the GRCA and measured in the field in August 2012.

Parameter	Rate loss [µg TAN L^{-1} m^{-1}]	Total loss [mg TAN L^{-1}] in 5700 m
Average	0.22	1.28
Median	0.23	1.32
± Std. Dev	0.04	0.2
Min - Max	0.16 - 0.26	0.93 - 1.5

The observed TAN loss rates corrected for dilution and adjusted by travel time (flow velocity=0.3 m/s) representing ammonium uptake, nitrification and ammonia volatilization downstream of the KTP, were expected to be higher that the estimated ammonia volatilization loss rates (in g N/m with fixed flow velocity 0.3 m/s and average river depth 0.50 m). However, the estimated volatilization rates were equal or greater than the net ammonium decrease observed in the Grand River downstream of the KTP on the plume sampling campaigns during the summer of 2010 and 2012 (Figure 4.12). This observation supports the assumption that the calculation done for the Grand River with grab samples overestimated the ammonia flux in the fringes of the plume and the water surface outside of the plume of nutrients.

Despite the fact that only one daytime sample was collected, the ammonia volatilization loss rate was quite stable along the day (dashed line in Figure 4.12). The observed TAN loss rate during the night showed an ample variability (solid line Figure 4.12) and indistinguishable from one another. Part of the variability could be attributed to variable biological demand of ammonium and nitrification.

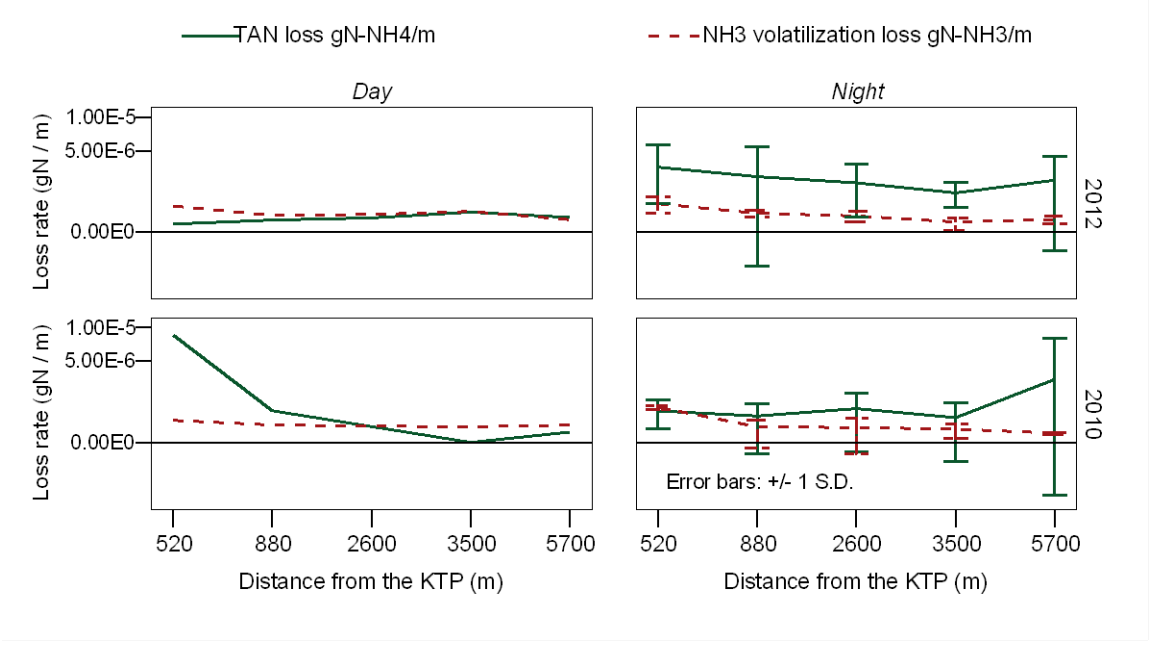


Figure 4.12 - Ammonia volatilization rate (*dashed line*) and ammonium decrease rate observed in the Central Grand River (*solid line*) in gram of nitrogen per meter. Loss rates estimated with concentrations chloride-corrected for dilution and adjusted by travel time (flow velocity=0.3 m/s). Day time sampling was done between 13:00 and 16:00 hours; night time sampling between 19:00 and 4:00 hours.

Observed nitrate concentrations below the KTP

Before upgrades to the KTP, nitrate concentrations in the KTP effluent were higher in 2011 and 2012 (4.1 and 5.3 mgN-NO_3^-/L respectively), compared to 2010 (1.6 mgN-NO_3^-/L; Tukey-Kramer HSD q=2.741), but discharge was similar, indicating that the water chemistry of the effluent was variable during all the sampling campaigns, and possibly throughout the year. After the KTP upgrades were completed, in-plant nitrification promoted an increase in concentration of nitrate discharged into the Grand River (22 ±5 mgN-NO_3^-/L), observed as increases in concentrations between 1 and 3600 m downstream the KTP, as a result of ammonia oxidation (≈ 4 mgN-NO_3^-/L Figure 4.13).

The variability observed within and among years is the result of variable volume discharge from the KTP and the differences in base-flow. The reduction in nitrate concentration at Blair is possibly the result of a well-mixed water column and denitrification. Compared to background concentration at 5000 metres upstream of the KTP (Bridgeport, 1.9 to 2.3 mg N-NO_3^-/L), there were increases in nitrate concentration in 2010 (2.8 to 3.2 mg N-NO_3^-/L), 2011 (2.2 to 2.8 mgN-NO_3^-/L), 2012 (4 to 3 mg N-NO_3^-/L) and 2013 (3.7 to 3.3 mg N-NO_3^-/L).

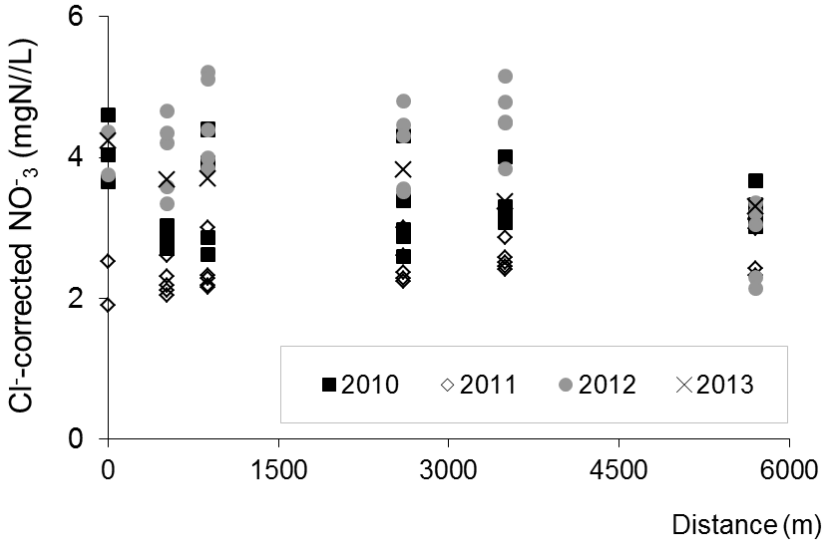


Figure 4.13 - Observed nitrate concentration (chloride-corrected for dilution) with distance within the plume of nutrients developing downstream of the Kitchener wastewater treatment plant. The centre of the plume is the location with the highest electrical conductivity.

However, when comparing only locations downstream of the KTP, there was a decrease in nitrate concentration in 2012 and 2013. That is to say, nitrate increases occurred during average or low flow years and decreased during the above-average base flow observed in 2013, despite the increases in nitrate from the KTP and are likely due to dilution. The increase in nitrate concentration observed during 2012 relative to other years is attributed to the observed below-average base flow (*i.e*. reduced dilution) and variable quality of the effluent due to the gradual changes in operation of the KTP (Region of Waterloo, *pers.comm*).

Table 4.6 - NO_2^-+NO_3^- increase rate constant before (2010-2012) and after (2013) Kitchener wastewater treatment plant upgrades. Rate constant *k* in s^{-1}, data corrected by travel time (provided by GRCA and in-field estimated). Negative rates represent a losing trend in NO_2^-+NO_3^-.

Year	Round	Rate constant *(k)*	r^2	Comments
2010	Evening	$0.15x10^{-3}$	0.52	Base flow (11m^3/s) similar to historical normal. Few locations appeared to be outside the plume. Increasing trend.
	Night	$0.92x10^{-4}$	0.72	
	Midnight	$0.74x10^{-4}$	0.71	
	Dawn	$0.68x10^{-4}$	0.63	
	Noon	$0.67x10^{-4}$	0.51	
2011	Evening	$0.88x10^{-4}$	0.87	Base flow (7 m^3/s) below historical normal. Data better constrained, increasing trend.
	Night	$0.62x10^{-4}$	0.68	
	Midnight	$0.88x10^{-4}$	0.98	
	Dawn	$0.11x10^{-3}$	0.93	
	Noon	$0.66x10^{-4}$	0.8	
2012	Evening	$0.61x10^{-4}$	0.43	Base flow (8 m^3/s) below historical normal. Large dispersion due to differences in KTP discharge. Apparent overall decrease.
	Night	$0.17x10^{-3}$	0.93	
	Midnight	$-0.78x10^{-4}$	0.52	
	Dawn	$0.11x10^{-3}$	0.8	
	Noon	$-0.11x10^{-3}$	0.68	
2013	Noon	$-0.31x10^{-4}$	0.42	Base flow (12 m^3/s) above historical normal. Greater nitrate release, dilution appeared to be the main decrease factor.

Changes in nitrate concentration during summer, low flow conditions fit first order equation $[NO_3^-]=[NO_3^-]_0 \cdot e^{-kd}$, with *d*=distance in metres (Table 4.6).The two negative rate constant *(k)* found (2012 and 2013) are interpreted as nitrite disappearing from the water column. The

negative rate constant observed in 2013 is assumed to be mainly due to dilution, given that 2013 was a year with above-historical normal discharge. No areal rates were calculated.

Nitrification estimates from nitrate increase

One of the processes associated with the decrease of ammonium along the river is nitrification. Nitrification rates can be estimated from nitrate increases, assuming that no nitrate uptake occurs, and that nitrate increases represent solely oxidation of ammonium coming from the KTP, negligible ammonification of mineralized organic matter in the river and no external inputs. Nitrate concentration in-river was greater than or equal to 1.7 mgN-NO_3^-/L at a sampling location above the KTP effluent (even higher during fall and winter). Part of this nitrate is from agricultural, non-point sources in the upstream area of the watershed; in addition to other wastewater treatment plants upstream the Region of Waterloo.

Nitrate concentration measured upstream of the plume was subtracted from the NO_3^- concentrations downstream of the KTP in order to measure the increase in NO_3^- within the plume as a result of the KTP effluent. By using the N-NO_3^- mass (adjusted by travel time) and estimating only transformation within the plume of nutrients, Figure 4.14 shows that there could be both net gain and loss of NO_3^- in the Grand River below the KTP, and that rapid nitrate production was sometimes observed during daylight hours as a result of nitrification. The rates typically ranged from -0.1 to 0.3 μmol N-NO_3^- m^{-1}. NO_3^- gains and losses are the result of ammonium oxidation and nitrate uptake respectively, in addition to the variable quality of the effluent discharged from the KTP.

Denitrification could account for some of the nitrate decrease observed in the Central Grand River, particularly during the nighttime, but also during the day, observed by Rosamond et al (2011) in July as a daytime peak in N_2O in some location in the Grand River. Denitrification removed as much as 44% of the total nitrogen in-stream in a north China Plain (Wang *et al*. 2011). Denitrification in the Grand River has been reported to be from 2 to 11%, depending the travel time (Rosamond 2013).

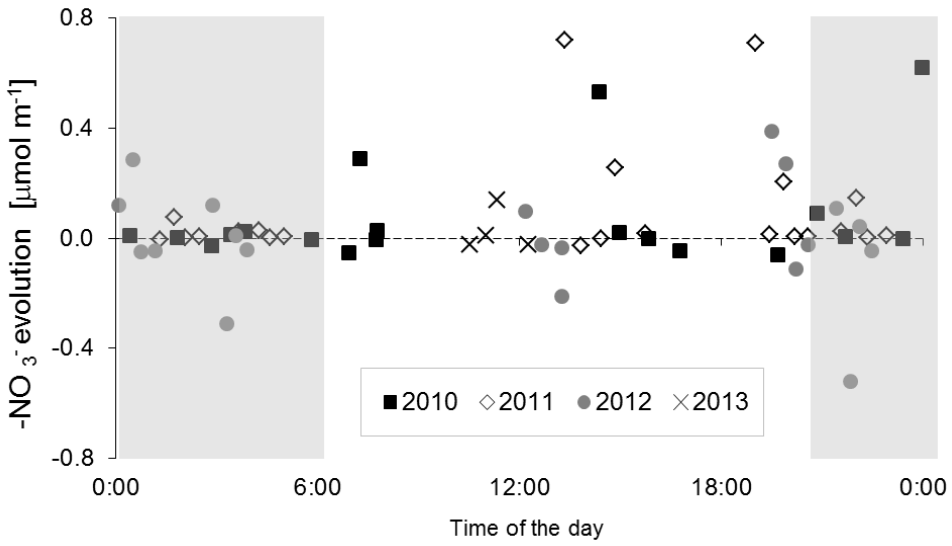


Figure 4.14 - Nitrate evolution (in µmol N-NO_3^- m^{-1}) by year within the plume of nutrients below the Kitchener Wastewater treatment plant. Shaded panels represent night hours. Rates calculated with chloride-corrected ammonium concentration adjusted by travel time (flow velocity=0.3 m/s).

Nitrate assimilation is assumed to occur simultaneously with photosynthesis in the daytime because NO_3^- reduction is energetically costly (Dortch 1990). Gammons *et al*. (2011) attributed decreases in DIN and phosphate to assimilation in a small stream. Given that TP showed a slight decrease downstream of the KTP (0.001 to 0.004 µgP m^{-1}) when dilution was accounted for, some of the nitrate released by the KTP is possibly being removed from the Grand River by assimilation.

The experiment presented in the Chapter 2 (*ammonium and nitrate assimilation*) suggested that epilithon assimilated nitrate at a very low rate (0.8 to 0.9 mgN m^{-2} h^{-1}) and ammonium at a high rate (4.8 to 7.3 mgN m^{-2} h^{-1}) and could account from at least 26% of the N removal. In a study completed in a river receiving 24000 m^3 of treated sewage per day in Japan, periphyton assimilation removed between 6 and 18% of the total nitrogen (Ogura *et al*. 2009). The Central Grand River received an average of 73195 m^3 per day (±12307 m^3 d^{-1}) during 2013; thus, a similar percentage contribution of periphyton assimilation to the TN decrease (between 6 and 18%) downstream of the KTP, would have accounted for a net N loss of 114-343 kg N d^{-1}.

Changes in dissolved inorganic nitrogen (DIN)

The production of nitrite (NO_2^-) was observed to be almost continuous in the 5700 m downstream of the KTP, due to the fact that nitrite is an intermediate species during the oxidation of ammonia into nitrate. There were slight differences between day and night (Figure 4.15) and nitrite evolution seem to decline at 5700 m during dry years (2011 and 2012). Almost no nitrite was observed after the KTP upgrade (0.19 mg N-NO_2^-/L at 520 m)

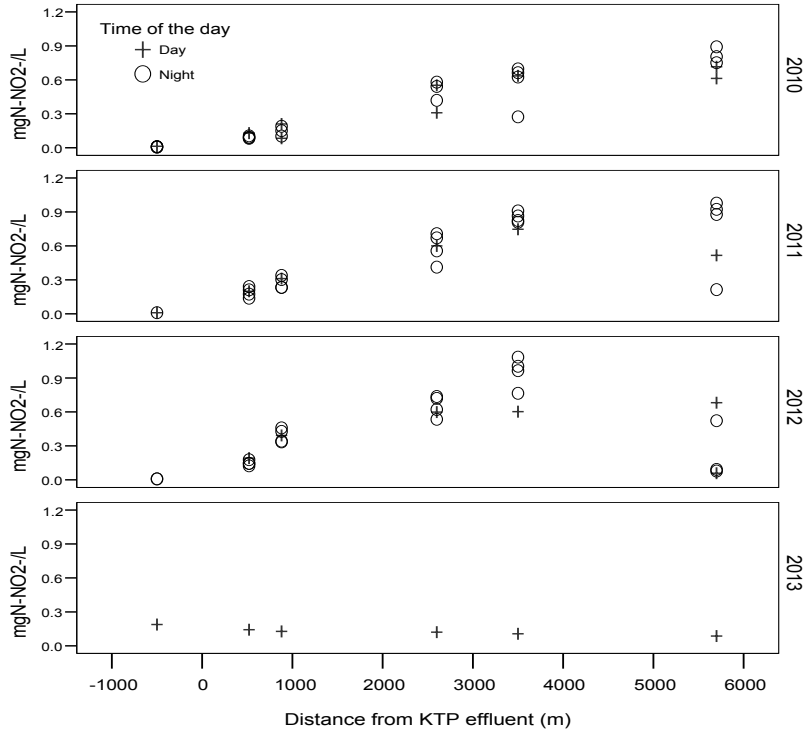


Figure 4.15 – Nitrogen-nitrite concentration (NO_2^-, mg N/L), corrected for dilution downstream of the Kitchener Wastewater Treatment plant before (2010-2012) and after (2013) upgrades. Day samples collected between 13:00 and 16:00; night samples collected from 19:00 to 4:00.

Changes in the nitrate concentrations downstream of the KTP are the result of ammonium oxidation before upgrades and increased nitrate discharge from the KTP after upgrades. Before upgrades, this trend was not expected due to the estimated high contribution from ammonia volatilization, when ammonium was in high concentrations in the river. However, the DIN concentration (NO_3^-+NO_2^-+NH_4^+) always increased downstream of the KTP compared to upstream concentrations (5000 m upstream of the KTP).

The DIN concentration in 2012 decreased from the KTP to 5700 metres downstream of KTP, but it did not return to background conditions for the 5700 metres sampled in this research. The DIN in the Central Grand River immediately downstream of the KTP (520 m) is almost doubled after receiving the effluent from the KTP (Figure 4.16). There is some N removal between the location closest to the effluent discharge (520 m) and the location at 5700 m below the effluent. This decrease in DIN is assumed to be the result of ammonia volatilization, nitrate uptake (as observed in Chapter 2) and moderated denitrification (approximately 11% nitrate removal in the Grand River, Rosamond 2013)..

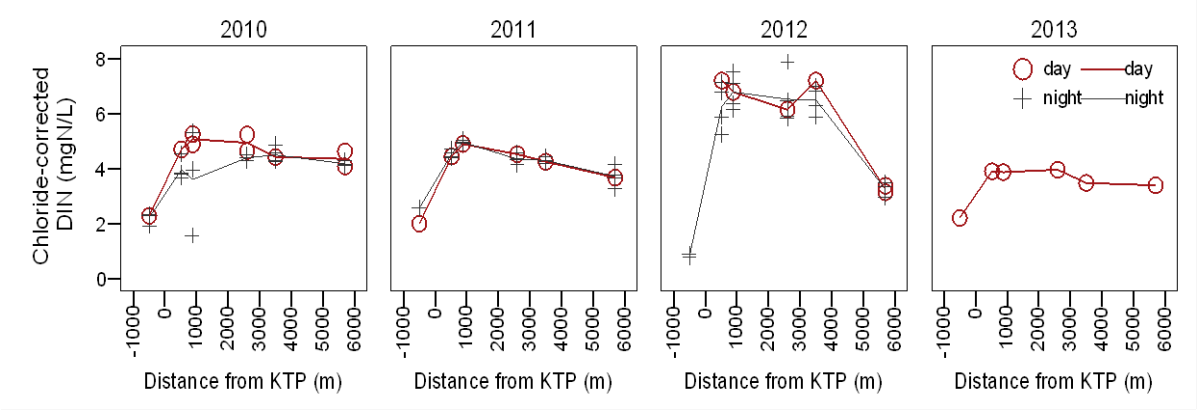


Figure 4.16 - Dissolved inorganic nitrogen concentration (NH_4^++NO_2^-+NO_3^-, mg N/L), corrected for dilution downstream the Kitchener Wastewater Treatment plant before (2010-2012) and after (2013) upgrades. Day samples collected between 13:00 and 16:00; night samples collected from 19:00 to 4:00.

Inter-annual differences in NO_3^- concentration could be attributed to different base flow among years, given that the KTP nitrogen discharge is relatively consistent year-round (Region of Waterloo, *comm. pers*). DIN flux estimates showed that nitrogen was lost from the water column in all years, and followed the same patters as DIN concentrations. DIN decreases at the location 5700 m from the KTP represent that ammonium was at very low concentration (below the detection limit 0.05 mgN-NH_4^+) and nitrate uptake and denitrification occurred. It is important to stress that the DIN fluxes here presented might be overestimated, because samples were collected in the centre of the plume of nutrients, where nutrient concentrations are likely highest than in the fringes of the plume and close to the river banks.

Complementary to the analyses on concentrations, by measuring the isotopic composition of nitrogen ($\delta^{15}N$) in ammonium and nitrate, it is possible to corroborate and add information about the processes occurring downstream of the KTP effluent.

Nitrate and ammonium δ^{15}N

In addition to the changes in concentration above described, the nitrogen stable isotope delta of ammonium ($\delta^{15}N_{NH_4^+}$) and nitrate ($\delta^{15}N_{NO_3^-}$) observed before and after the KTP upgrades, provided additional information about the relative contribution of the processes occurring downstream of the KTP.

Before upgrades (2010-2011), ammonium comprised a large part of the dissolved inorganic nitrogen released from the KTP effluent, at concentrations higher than 20 mgN-NH_4^+/L and $\delta^{15}N_{NH_4^+}$ between +4 to +10‰. The $\delta^{15}N_{NH_4^+}$ increased downstream, from +5‰ as high as +30‰ in 2010 as the ammonium concentration decreased from 2 to around 0.5 mgN-NH_4^+/L (Figure 4.17). After upgrades (2013), the ammonium concentration was low ($\leq$ 6 mgN-NH_4^+/L) as a result of more efficient ammonia oxidation, and the isotope delta of the KTP effluent was already enriched in ^{15}N (≈23‰) due to the more efficient ammonium oxidation and volatilization inside the wastewater treatment plant. Decreases in concentration and increase in the $\delta^{15}N_{NH_4^+}$ has also been observed in small eutrophic streams (Gammons et al. 2010), estuaries of large rivers with urban and farm inputs (Cifuentes *et al*. 1989) and rivers in semiarid regions (Segal-Rozenhaimer *et al*. 2004).

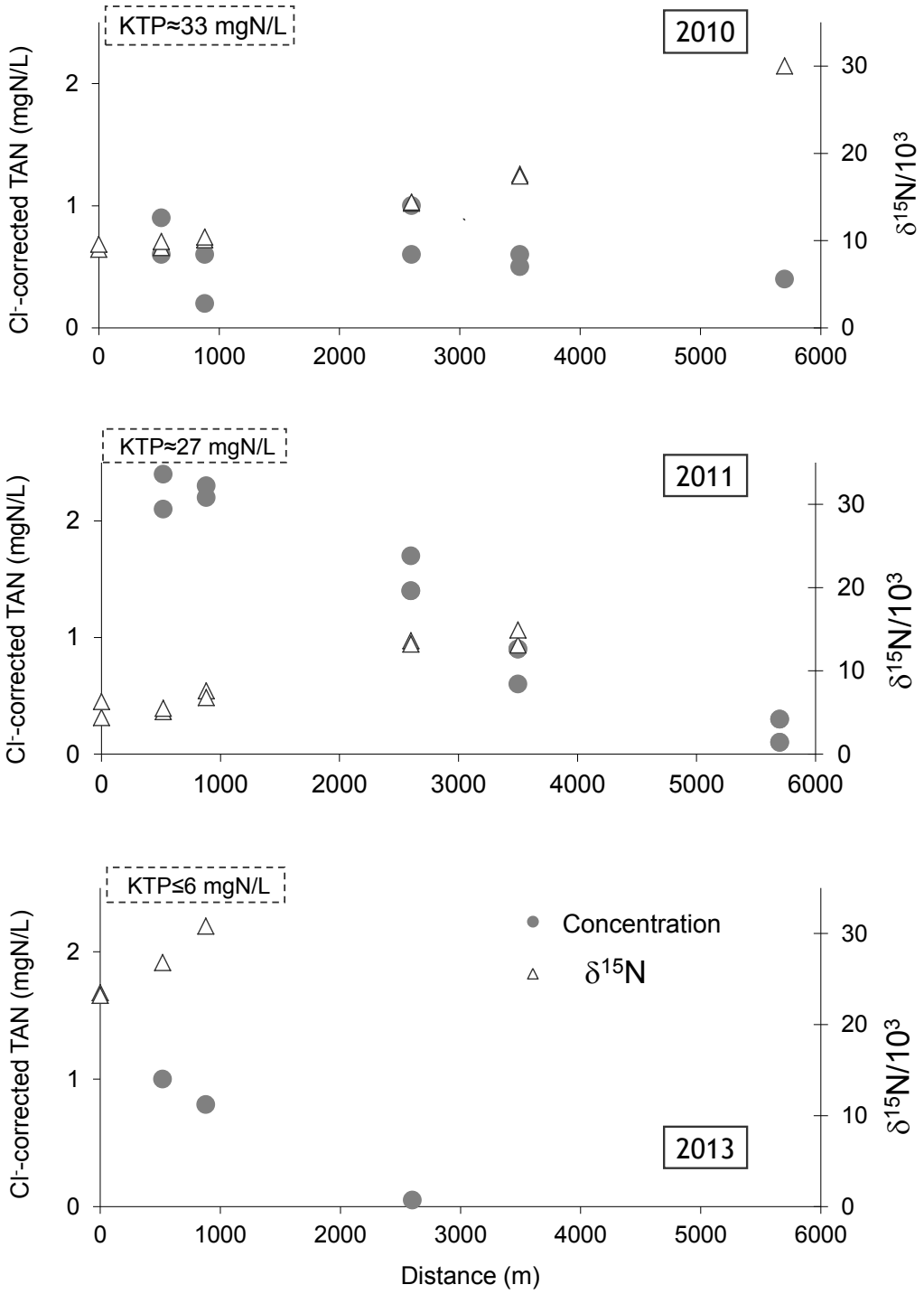


Figure 4.17 - Ammonium chloride-corrected concentration (●) and nitrogen isotope delta (∆) in the Central Grand River downstream the Kitchener wastewater treatment plant before (2010-2011) and after (2013) upgrades. Note that the ammonium concentration from Kitchener wastewater treatment plant (0 m) is too high to be shown. Analytical precision ±0.3‰ for ^{15}N.

Differences in the day and night $\delta^{15}N_{NH_4^+}$ were not observed before upgrades (Figure 4.18). Such comparison is not possible after upgrades at the KTP because samples were collected during the day only. Ammonia volatilization strongly fractionates N isotopes (Robinson 2001) and it is a process that leaves the remaining N enriched (Högberg 1997). It is important to stress that ammonium assimilation and nitrification can also contribute to the ^{15}N enrichment observed in the ammonium; however, the relative stable volatilization rates calculated in day and night together with the consistent ^{15}N enrichment with distance, suggest that before the KTP upgrades, ammonia volatilization likely was the process contributing the most to the differences in the ammonium isotope delta in the central Grand River. Modeling (described later) was also performed to evaluate the main processes affecting the concentration and isotopic composition of ammonium.

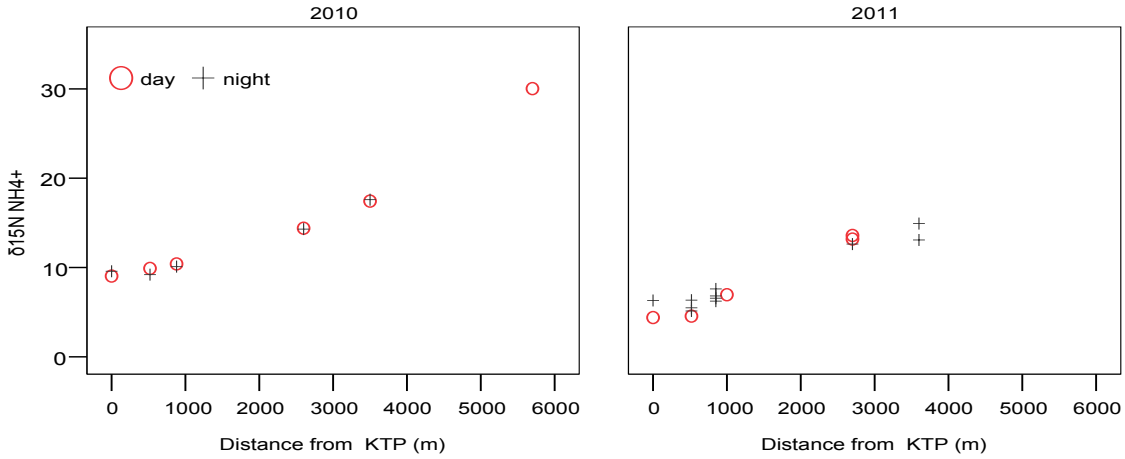


Figure 4.18 – Ammonium isotope delta ($\delta^{15}N_{NH_4^+}$) before upgrades in the Kitchener wastewater treatment plant (2010 and 2011). Day time sampling from 13:00 to 16:00; night time sampling from 19:00 to 4:00.

The $\delta^{15}N_{NO_3^-}$ upstream of the KTP varied from +8.5 to +8.9 ‰ in winter (3.5 to 5 mgN-NO_3^-/L) to +10 to +15‰ in summer and fall (1.5 to 3.8 mg N-NO_3^-/L). High nitrate concentrations above the KTP effluent during the cold part of the year was observed before and after the upgrades, and likely represents NO_3^- from agricultural areas located in the northern part of the watershed (manure $\delta^{15}N \approx 12$‰ according to Kellman and Hillaire-Marcel 1998, 2003).

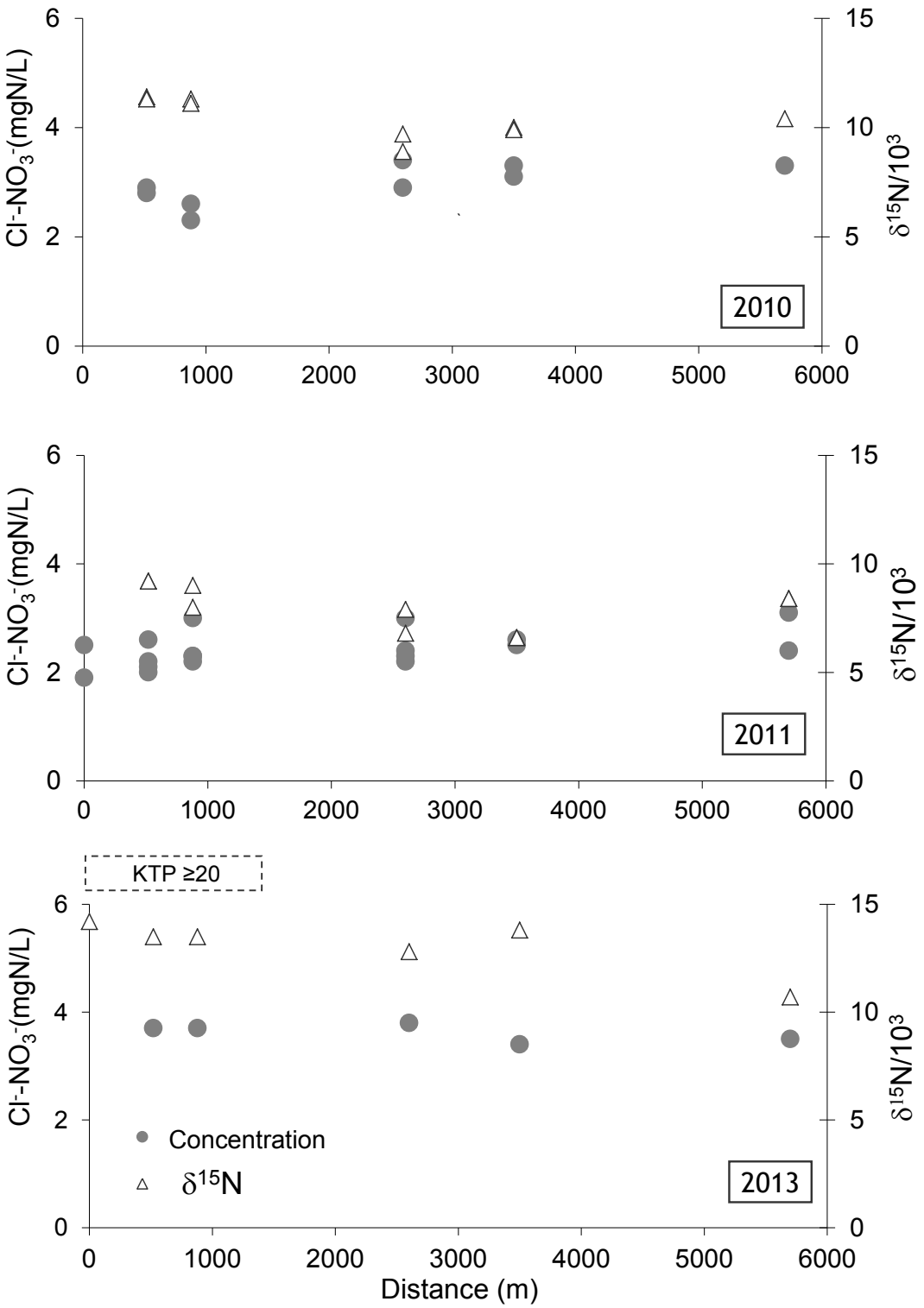


Figure 4.19 - Nitrate chloride-corrected concentration (●) and nitrogen isotope delta (∆) in the Central Grand River downstream the Kitchener wastewater treatment plant before (2010-2011) and after (2013) upgrades. Note that the nitrate concentration from the Kitchener wastewater treatment plant (0 m) is off of the axis. Analytical precision ±0.3‰ for ^{15}N.

Additionally, other WTP's (Waterloo, Elora, Fergus) discharged in the Grand River upstream of the KTP. Therefore, the $\delta^{15}N_{NO_3^-}$ observed in the Grand River varied seasonally, represented several nitrate sources and likely modified the $\delta^{15}N_{NO_3^-}$ downstream of the KTP as a result of mixing with the nitrate in the effluent and the nitrate being produced in the water column.

Before the upgrades, the KTP effluent had a $\delta^{15}N_{NO_3^-}$ from -6 to +1‰ at concentration between 2 and 4 mgN-NO_3^-/L. After the upgrades, the nitrate isotopic composition measured in the effluent varied between +4 and +11 ‰ at concentrations above the 20 mgN-NO_3^-/L. Thus, compared to pre-upgrades conditions, the ^{15}N-enriched ammonium is the substrate for the slightly ^{15}N-enriched nitrate produced inside the KTP (Figure 4.19).

The $\delta^{15}N_{NO_3^-}$ of the Grand River water downstream of the KTP before the upgrades showed a slight depletion trend with a small increase in nitrate concentration, suggesting that nitrification was occurring (Figure 4.19). A similar range in $\delta^{15}N_{NO_3^-}$ (+6 ‰ to +11 ‰) has been observed in streams receiving effluent from a WTP (Gammons et al. 2010). In the Central Grand River, dilution with groundwater is low compared to the Central South reach of the Grand River between Paris and Brantford (Lake Erie Region Source Protection Committee 2012; Westberg 2012); thus, mixing of masses of water with different $\delta^{15}N_{NO_3^-}$ was not expected. One other possibility for the relative stable trend in NO_3^- concentrations and $\delta^{15}N_{NO_3}$ is that nitrate gains and losses are about equal (nitrification ≈ nitrate assimilation + denitrification), thus the changes in concentration and $\delta^{15}N_{NO_3^-}$ are expected to be small (Mariotti *et al*. 1988) assuming that nitrate assimilation did not have a large impact in the nitrate isotope delta.

Modeling rates of processes with nitrogen stable isotopes

The changes in isotope delta of ammonium are the result of ammonia volatilization, nitrification and ammonium uptake. A box-model developed by Venkiteswaran *et al*. (2011) uses ammonium and nitrate concentrations and isotopes of both ammonium and nitrate for estimating the rates of these processes in rivers. Field-determined pH and temperature data are used to partition ammonium and ammonia (*i.e* initial conditions). The model was created in Matlab and can be used to do forward simulations and, when run inversely, to find best-fit

solutions for field data. To find best-fit solutions, the model selects initial conditions based on the field data and iteratively adjusts the rate constants to find a best solution that minimizes the sum of squared errors (SSE) between field data and model outputs. In Matlab this is done with the *fminsearch* function and is a form of non-linear optimization.

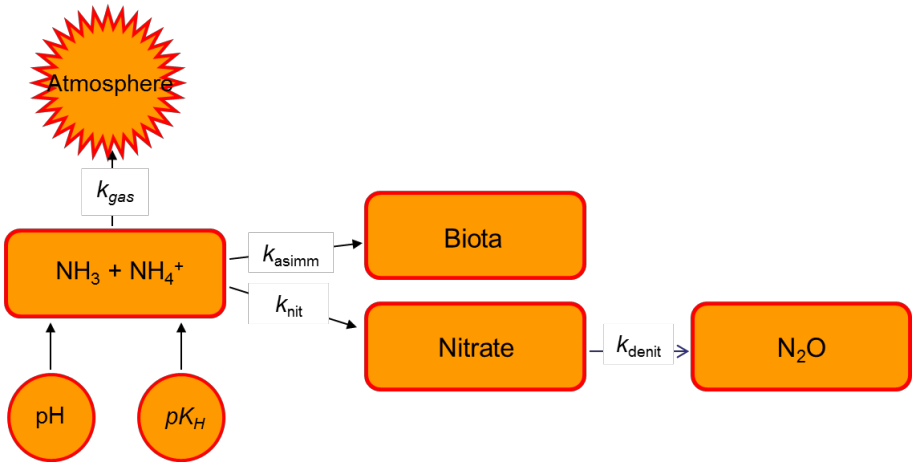


Figure 4.20 - Conceptual model of ammonium and nitrate in the Grand River. Rate constants: volatilization (k_{gas}), assimilation into biota (k_{assim}), microbial oxidation (k_{nit}) and denitrification (k_{denit}). Image (modified) and model created by J.J. Venkiteswaran *et al. (2011).*

This model is driven by the gas exchange coefficient for volatilization (k_{gas}) and first-order rate constants for microbial ammonium oxidation (k_{nit}), assimilation of ammonium into biota (k_{assim}) and denitrification (k_{denit}, Figure 4.20). Table 4.7 shows the physical-chemical parameters and fractionation factors used for the model . The alpha values used in Table 4.7 were taken from different published literature and . That is why table 4.10 was presented, in order to show the changes in alphas.

The model provided first order rate (m^{-1}) and the change in concentration that shows how much ammonium was moved through each process (Table 4.8). The results of the model shows that the rate constant for gas exchange (*i.e*. volatilization) and ammonium assimilation were similar before upgrades (gas exchange in 2011 was half of estimated rate). However, the change in concentration was larger for ammonium assimilation downstream of the KTP (Table 4.8), presumably due to the large amount of biomass in the river. Isotope data for 2012 were not

analysed due to the oscillations of the quality of the effluent; thus was not modelable given that the concentration-isotopic composition data by sampling location was not available.

After upgrades (2013) the rate constants for gas exchange and ammonium assimilation were similar to those estimated before upgrades; however nitrification appeared to occur at a slightly faster rate (one order or magnitude higher than before upgrades). The rates estimated for each process in the model likely changed due to the ammonium preference over nitrate and reduced ammonia volatilization due to the reduced amount of free ammonia ($NH_{3\ aq}$).

Table 4.7 - Data and source of physical-chemical parameters and fractionation factors (α) used for the model. Bolded terms used in the model are from several sources on different field and experimental conditions.

Physical-chemical parameters

a. $pK_a = \frac{2533}{(T + 273.15) - 0.5936 \times \log(T + 273.15) + 4.127}$; T in°C (Olofsson (1975)

b. Henry's Constant: log K_H = 1.7709

Fractionation factors

a. *NH_3 dissolution*: Li et al. (2012) 1000•ln(a):

$$\alpha_{NH3aq} = \frac{1}{\exp\ (25.94 \times \frac{10^3}{T°C + 273.15} - 42.25)}{10^3}$$

b. *Kinetic gas exchange fractionation at 25°C*: Kirschenbaum et al. (1947), Thode et al. (1945) and Norlin et al. (2002): **α_{gas}= 0.995**

c. *Nitrification*: Gammons et al. (2010), Delwiche and Steyn (1970); Mariotti et al. (1981); Casciotti et al. (2003); including experimental and empirical results, night- and day-time periods, variable temperature: **α_{nitn}= 0.974**

d. *Ammonium assimilation*: Delwiche and Steyn (1970); Mariotti et al. (1981) and Fogel and Cifuentes (1993): **α_{assim}= 0.996**

e. *Denitrification*: fractionation from 0.960 to 0.995 (Kendall 1998 and Sebilo et al., 2003): **α_{denit}= 0.985**

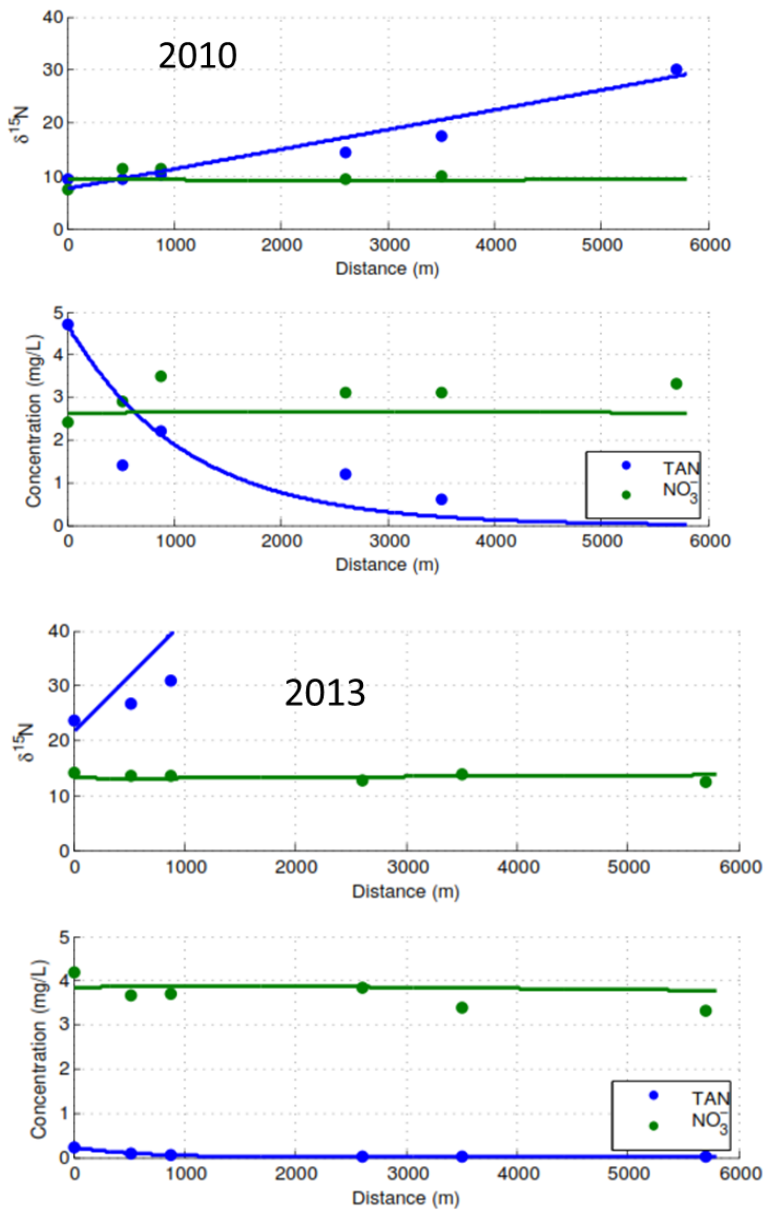


Figure 4.21 - Modeled result of ammonium (TAN) and nitrate (NO_3^-) isotopic composition (*upper panels*) and concentrations (*lower panels*) downstream of the Kitchener wastewater treatment plant before (2010 and 2011, *above*) and after (2013, *below*) upgrades during summer, low flow conditions. Model developed by Venkiteswaran et al. (2011)

Table 4.8 - Modeled first order rates (*k*, in m^{-1}) and change in concentration (in mg $N\text{-}NH_4^+$/L or mg $N\text{-}NO_3^-$/L) for the section comprising 5700 m of the Grand River downstream of the Kitchener wastewater treatment plant effluent. Negative gas exchange represents evasion to the atmosphere. Data represent before (2010 and 2012) and after (2013) KTP upgrades. Model developed by Venkiteswaran *et al.* (2011). TAN_{init} is the initial NH_3+NH_4^+ used for the model (estimated from instant chloride dilution at 1 m downstream the effluent. Refer to *Plume delineation* section for details.

Year	***2010***	***2011***	***2013***
TANinit	4.72	4.57	0.23
kgas	8.09 x10-4	4.63 x10-4	8.03 x10-4
knit	1.09 x10-5	2.56x10-5	7.75x10-4
kassim	8.99 x10-4	7.55 x10-4	9.73 x10-4
kdenit	2.66 x10-6	2.71 x10-6	1.39 x10-6
Concentration gas exchange	-0.2276	-0.134	-0.0081
Concentration ntrification	0.0538	0.1447	0.0984
Concentration NH4+assimilation	4.393	4.259	0.123
Concentration denitrification	0.0409	0.037	0.304

Table 4.9. Coefficient of determination (R^2) and root mean square deviation (RSME) for the ammonium (NH_4^+) and nitrate (NO_3^-) concentrations and isotopes for the model fit before (2010 and 2012) and after (2013) the Kitchener wastewater treatment plant upgrades. Model developed by Venkiteswaran *et al.* (2011).

		NH_4^+	NO_3^-	$\delta^{15}N_{NH_4^+}$	$\delta^{15}N_{NO_3^-}$
2010	R^2	0.8155	0.4676	0.9377	0.2702
	RMSE	0.4204	0.1743	0.1254	0.1466
2011	R^2	0.8832	0.3447	0.9719	0.0188
	RMSE	0.3244	0.089	0.0706	0.1113
2013	R^2	0.9821	0.144	0.9755	0.333
	RMSE	0.178	0.088	0.1557	0.0541

Compared to the model (4% ammonium removal due to volatilization and 92% ammonium removal due to assimilation), the results obtained in this thesis suggest that ammonia volatilization (50% ammonium removal, see *Estimates of ammonia volatilization* in this

chapter) was more important than ammonium assimilation downstream of the KTP effluent before the upgrades (20% ammonia removal, see *Epilithon contribution to nitrogen cycling downstream of the KTP* in Chapter 3). However, it is important to emphasize that the instantaneous estimation of ammonia volatilization was done with samples collected inside the plume of nutrients, the section of the river with the highest ammonium concentration. Thus, when estimating the ammonia flux by surface area in the fringes of the plume and in the rest of the Grand River (outside of the plume) likely there was an overestimation of the vertical flux.

The second important difference between the modeled results and the estimates proposed in this thesis is related to ammonium assimilation (20% removal in experimental conditions, 92% removal modeled assimilation). Ammonium assimilation was measured in experimental conditions and estimated only epilithic assimilation. The epilithon biomass measured in experimental conditions was 73 g/m^2 (±37 g/m^2); whereas Hood (2012) estimated the macrophytes biomass downstream of the KTP in around 300 g/m^2. Thus, the calculated ammonium (and nitrate) uptake described in chapter 2 represents only epilithic uptake; the total uptake by all biotic component of the Grand River (macrophytes, epiphyton and plankton) would be likely greater that the rates reported in this thesis and possibly similar in importance to ammonium assimilation rates obtained by the Matlab model due to the great amount of biomass existing in the Central Grand River downstream of the Kitchener wastewater treatment plant.

The model provided constant rates simultaneously estimated for the three processes removing ammonium in the Grand River downstream of the KTP effluent during summer low flow. In comparison, the approach used in this thesis provided experimental evidence of ammonium assimilation and estimates of ammonia volatilization. Moreover, the isotopic fractionation due to ammonia volatilization was also obtained in experimental conditions and the isotopic fractionation factor due to assimilation is proposed by the isotopic separation of the source (ammonium) and the product (biomass). Additionally, the results of the experiment of ammonium assimilation in epilithon (Chapter 2) are largely variable across concentrations. Thus, the most important differences between the modeled results and the proposed contribution by processes presented in this thesis are related to the experimental design, the

potentially overestimated volatilization, the assimilation rates measured for epilithon only and the propagation of the error between the processes contributing to ammonium decrease, making difficult to differentiate among processes.

Additional variability originates from the ample range in fractionation factors (α) reported in the literature. Previously reported isotopic fractionation during ammonium assimilation oscillates between 1.002 and 1.012 (Högberg 1997; Robinson 2001 and Chapter 5 in this thesis); the isotopic fractionation during nitrification has been reported from 1.010 to 1.0as high as 1.025 (Mariotti *et al*. 1981; Högberg 1997; Evans 2007; Gammons *et al*. 2010) and the fractionation due to ammonia volatilization was experimentally measured in 1.019 in water from the Grand River (Chapter 3, this thesis). The Matlab model assumed fixed fractionation factor values that reduced the error but constrained the possible results to the conditions provided. The Table 4.10 shows the overall isotopic fractionation factor (estimated form an isotopic mass balance) for three potential different scenarios, the Matlab model and the data observed in the Grand River downstream of the KTP before upgrades.

Table 4.10 - Isotopic fractionation factors (α) and fraction of ammonium removal (*f*) by ammonia volatilization (*volat*), ammonium assimilation (*assim*) and nitrification (*nitrif*) in the Central Grand River downstream of the Kitchener wastewater treatment plant effluent during summer, low flow conditions before upgrades (2010-2012). The case *Observed Field* uses same fractionation factor as Matlab model and the fraction removed by each process (*f*) as proposed in this thesis. Volatilization and assimilation has the same ammonium removal fraction due to the difficulty or distinguishing between processes.

Case	α_{volat}	f_{volat}	α_{asimm}	f_{assim}	α_{nitrif}	f_{nitrif}	$\alpha_{overall}$
1	1.019	0.5	1.010	0.3	1.015	0.2	***1.016***
2	1.019	0.4	1.002	0.4	1.010	0.2	***1.010***
3	1.019	0.3	1.002	0.5	1.010	0.2	***1.009***
Matlab	1.005	0.04	1.004	0.92	1.025	0.04	***1.005***
Observed Field	1.005	0.45	1.004	0.45	1.025	0.1	***1.007***

The cases 1 to 3 included the isotopic fractionation factors and the mass removal proposed in this thesis (see Table 4.10). The Matlab model used the values provided in Table 4.7, and the *Observed Field* represents the best fit to the observed $\delta^{15}N_{NH_4^+}$ using the equation $\delta^{15}N_t=\varepsilon \ln f_t$.

The fractionation factor with the best fit to the observed $\delta^{15}N_{NH_4^+}$ before upgrades (2010 and 2011) was 1.006 and 1.007 after the KTP upgrades (2013)

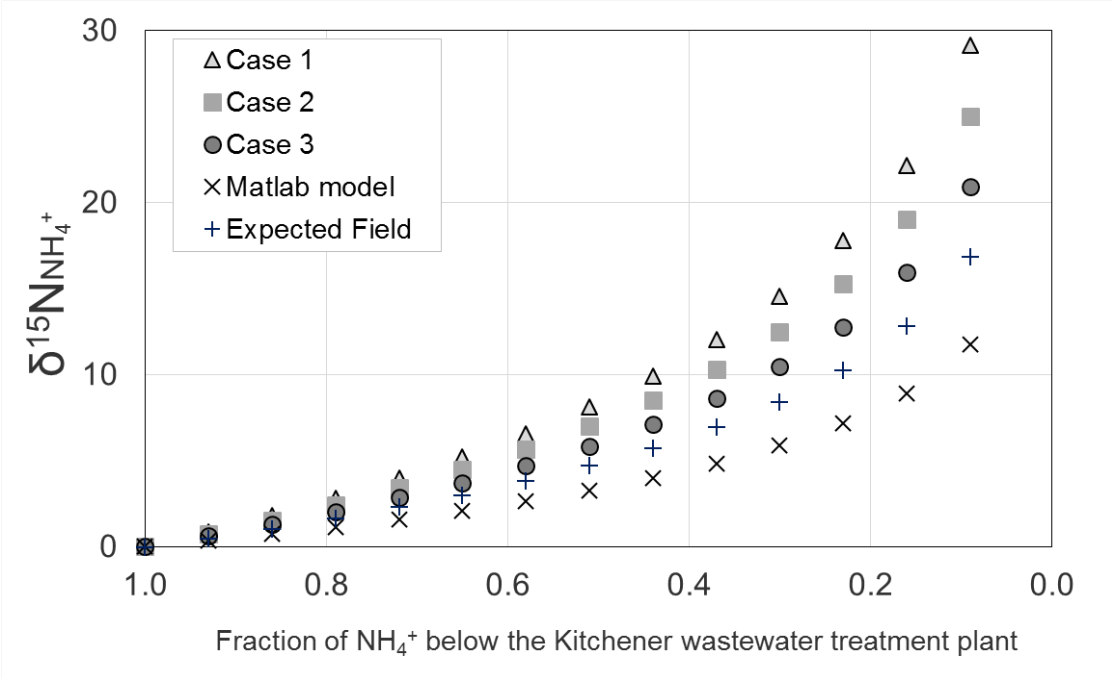


Figure 4.22 - Variability in the ammonium isotope delta estimated from an isotope mass balance in the Central Grand River downstream of the KTP. Ammonia volatilization, ammonium assimilation and nitrification are the process causing the ammonium decrease. The *X* axis represents the remnant fraction of ammonium in the water column as it flows downstream. Matlab model by Venkiteswaran *et al.* (2011). The Observed Field curve was fit to the observed $\delta^{15}N_{NH_4^+}$ using the equation $\delta^{15}N_t = \varepsilon \ln f_t$.

The Figure 4.22 shows the isotopic mass balances expected due to the different fractionation factors and variable contribution of ammonia volatilization, ammonium assimilation and nitrification showed in table 4.10. The Figure 4.22does not match the observed field values due to the fact that the observed in the field curve uses the same alpha values as the Matlab model, but changes the fraction of ammonium removal to the values, giving equal importance to volatilization and assimilation (45% each) and 10% ammonium lost due to nitrification. The example cases (1 to 3) had isotope alphas values and fractional contribution by process from previously published literature and the experimental value obtained in this thesis for volatilization ($\alpha_{volatilzation}$=1.019, see Chapter 3). The main assumption for figure 4.22 is that the overall isotopic fractionation factor observed in the field could oscillate inside the values provided in table 4.10. It is likely that the ammonium removal processes do not occur at the

same rate; thus, the fraction *f* represents the "magnitude" of ammonium removal attributed to each process within reasonable boundaries. I consider that it is not possible to provide an effective or *true* match between the hypothetical cases and the observed in field because there is high uncertainty about the *real* alpha values and the contribution by process in the Grand River. In addition, some sections of the river would have assimilation more intense than nitrification; which complicates the calculations proposed for the section of the central Grand River downstream of the KTP effluent. Figure 4.22 shows that the assumed contribution and alpha by process were not exactly as proposed in Table 4.10 or that those values can change among years year; however, it provides an acceptable range of values that could be expected in field conditions.

It is likely that in cases 1 and 2, the contribution by process was overestimated and the fractionation factors represent extreme α values. The overall fractionation factor observed in the Grand River downstream of the KTP before upgrades (estimated from the isotopic mass balance) was between the case 3 and the Matlab model. Thus, for the result obtained in the Matlab model, a process with a small fractionation factor that moved a great amount of mass (*i.e* assimilation) resulted in a greater rate and had more influence in the model. Similarly, a process that moved few mass even with a large fractionation factor (nitrification) will not change too much the isotopic composition of the ammonium pool, suggesting that ammonium oxidation was not a very important process.

Calibration and validation of the model, or even the creation of a new model incorporating the data presented in this research would likely improve our understanding of the ammonia removal processes in the Grand River and could incorporate nitrate uptake rates and individual uptake rates and fractionation factors for different biotic components, in order to have a more complete nitrogen assimilation model for the Grand River downstream of N point sources.

Additional considerations

Designating the Central Grand River as a reach with impaired in-stream conditions due to wastewater discharge would largely depend on the particular goals of the final users and the indicators used to determine such conditions; namely drinking water standards, environmental

quality standards or critical toxicity values for individual compounds. The Grand River Conservation Authority (2012), estimated that upgrades to the KTP will result in an increase of nitrate of 1.1 mg L^{-1} immediately downstream of the plant, and that the impact will be ameliorated as the river flows south by nitrogen uptake and dilution with groundwater between Cambridge and Paris, downstream of the study area. In the summer of 2013, nitrate concentration in the KTP effluent was high ($\geq$ 20 mg N-NO_3^-/L; however, it was diluted due to the above-average base flow observed that year. Nitrate concentrations will increase in drier years, exacerbated as the population served by the KTP increases. Thus, the expected nitrate increase downstream of the urban area may be higher than modelled by the GRCA.

The KTP upgrades resulted in higher dissolved oxygen levels and no hypoxia (DO <2 mg/L) at the location Blair (5700 m below KTP). However, denitrification could be reduced as a result of the higher dissolved oxygen conditions in the water column, resulting in nitrate remaining in the Grand River further downstream and exported to Lake Erie if it is not assimilated.

The KTP upgrades promoted higher nitrate concentrations and lower phosphate. These changes in the quality of the effluent can also lead to changes in taxonomical composition of the community downstream of the KTP and its productivity. Carr *et al*. (2003) modeled the macrophyte biomass in south western Ontario (including the Grand River) and suggested that reducing nitrogen loading from WTP's would result in reduced macrophytes biomass. Chambers and Prepas (1994) found that changes in the aquatic macrophyte community at the Saskatchewan River were partially responsible for changes in the water chemistry downstream of a wastewater discharge site. Thus, nutrients and macrophytes affect each other and such changes should be measured in the Central Grand River. An increase in nutrients and decrease in base-flow could trigger changes in the plant community. Vis et al. (2007) found that 10% decrease in flow enhanced phytoplankton production and promoted filamentous algal mats. Increased fluxes of nutrients and decreased base-flow are likely to occur in the Grand River during dry years; thus, it is necessary to monitor extreme conditions in order to understand and adapt the WTP's operations to the overall water management at the watershed level.

The data collected in this study was constrained to the section of the Grand River impacted by the Kitchener Wastewater Treatment Plant, and included an assessment of the evolution of the water quality leaving a treatment plant before, during and after plant upgrades in summer, low flow conditions, when dilution is at its minimum and the effluent discharge represent the greatest impact into the Central Grand River. These data might be incorporated into nutrient models such as GRSM (*Grand River Simulation Model*) or NANI (*Net Antropogenic Nitrogen Inputs*), as well as future models developed for specific conditions and objectives. Finally, the effect of the Speed River, which enters the Grand River downstream of Blair and is significantly impacted by wastewater from the nitrifying Guelph treatment plant, was not evaluated and deserves special attention as the population served grows.

CONCLUSIONS

This study contain valuable information about the observed TAN decrease in the Central Grand River as a case study of a river receiving discharge from a wastewater treatment plant before and after upgrades in a WTP operation. To my knowledge, this is the first time that a before-and-after approach has been done in order to understand the nitrogen cycle downstream a wastewater treatment plant. It represents a case study of the effects of WTP upgrades in a urban temperate river in North America. Although the results obtained one year after the upgrades are limited, significant geochemical changes were observed downstream of the KTP. Further study could help complete the characterization of after effects, especially during low-flow years.
The plume of ammonium downstream the KTP before upgrades was found to reach further than 5700 metres during summer, low flow conditions. The ammonium decrease rates adjusted by travel time ranged from 0.03 and 0/69 $\mu gN\text{-}NH_4^+ s^{-1}$ at a flow velocity of 0.3 m/s. Before the KTP upgrades, the ammonia volatilization was estimated between 0.61 and 0.13 $\mu gN\text{-}NH_3/L$ per metre, or 0.18 to 0.04 $\mu gN\text{-}NH_3/L$ per second; which represents around 50 % of the TAN generated by the KTP. This is the first time that ammonia volatilization is estimated form a river receiving wastewater treatment plant effluent.

Despite the fact that discharge from the KTP had increased nitrate concentrations, net nitrate losses and net nitrate gains were observed in the Grand River, likely influenced by the variable summer low flow observed during the four years of the study. Downstream of the KTP, the

DIN did not return to upstream levels; however, the decrease in nitrogen at the location 5700 m downstream of the KTP suggest that nitrogen was removed from the water column nitrate assimilation denitrification in addition to the ammonia removed by volatilization and assimilation. The modeling results showed that ammonium assimilation was an important process which has an influence on the isotopic composition of the ammonium. The discrepancies between the experimental data and the modeling results are that assimilation was probably underestimated and ammonia volatilization overestimated. The modeling results provided constant rates estimated simultaneously for the three processes removing ammonium downstream of the KTP effluent; however, it used fractionation factors from the literature, which are highly variable and likely simplified the processes that affect the nitrogen cycling in the central Grand River. The observed $\delta^{15}N_{NH_4^+}$ and $\delta^{15}N_{NO_3^-}$ before the KTP upgrades suggested that ammonia volatilization, nitrification and NH_4^+ and NO_3^- uptake occurred in the Central Grand River downstream of the KTP. It is possible that ammonia volatilization was the process that contributes the most to the observed ^{15}N-enrichment in the $\delta^{15}N_{NH_4^+}$ observed in the Grand River downstream of the KTP before upgrades. After the upgrades, ammonia oxidation and (possibly) ammonia volatilization inside the KTP caused the observed $\delta^{15}N_{NH_4^+}$ ≈23‰. Higher $\delta^{15}N_{NH_4^+}$ also resulted in high $\delta^{15}N_{NO_3^-}$ in the river after the KTP upgrades as a result of nitrification of the remnant ammonium and mixing of nitrate with different isotopic composition.

Due to seasonal variations in river discharge and the expected changes in the quality of the effluent, the sampling efforts were focused on describing and explaining the influence of the plume of ammonium downstream of the KTP, the spatial effects in the aquatic ecosystem during summer low flow conditions. The sampling strategy used in this research was designed to assess the impact of WTP effluent on rivers with special emphasis in *i)* the extent of the plume of nutrient until it reaches certain regulatory limits, *ii)* adequate number of sample location and *iii)* the calculation of losses and gains of ammonium and nitrate by distance, without using hydrologic parameters. This last approach is especially useful in ungauged rivers or streams, or where no significant changes in flow occur. A different monitoring strategy should be used according to the goals of the user.

CHAPTER 5 - PERIPHYTON AS AN ENVIRONMENTAL ARCHIVE IN RIVERS RECEIVING WASTEWATER: THE CASE OF THE GRAND RIVER, ONTARIO, CANADA.

INTRODUCTION

Periphyton is a broad term applied to the microbiota established on living or inert substrata, such as wood, plants, rocks and even animals. Although etymologically imprecise, the term periphyton is broadly accepted and used (Wetzel 2001). Different names have been assigned to this community according to the substrate on which it grows: *epiphyton* upon aquatic plants, *epipelon* if established in sediments, *episammon* on sand, *epixylon* on wood and *epilithon* on rocks. A large part of the components of the periphyton derives from free-floating organisms (bioseston), particulate matter (abioseston) and sediments (Figure 5.1).

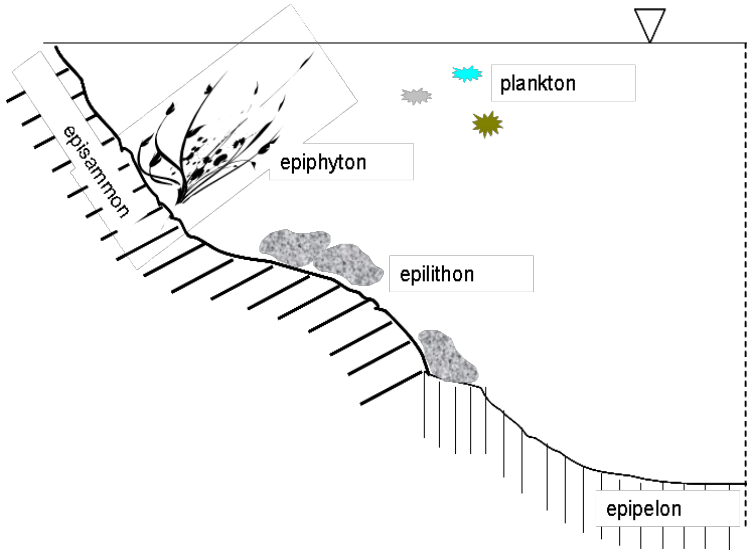


Figure 5.1 - Schematic view, cross-section of the Grand River, showing the periphytic communities and their typical spatial location.

The colonization of periphyton has been described as a gradual but continuous process, starting with deposition of organic substances, where bacteria begin to feed on dissolved and suspended particulate organic matter. Bacteria create mucilage that creates binding sites for all other organisms and materials. Diatoms appear to be the second taxonomic group adhering to the matrix. The climax stage is reached when filamentous algae establish a stratified community, where grazers, predators and scavenger concur. The taxonomic diversity of the community

changes through time as a result of grazing, detachment and sediment blasting, primarily influenced by changes in flow velocity (Azim and Asaeda 2005; Wetzel 2001).

The existence of autotrophic and heterotrophic organisms attached to different surfaces and submerged most of the time is likely to influence the nitrogen dynamics of the water column (Ogura et al. 2009). The nitrogen removal not only promotes biomass growth, but also provides information about the processes involved in the N transformations. Those processes impart a distinctive isotopic composition on the newly-formed biomass and on the nitrogen that was not assimilated or transformed by physical mechanisms or biological transformation accomplished by the components of the periphytic community.

Stable isotopes analyses in aquatic systems have been used to describe trophic levels, trophic networks and to differentiate organisms in different sections of a watershed (Loomer 2008). The use of nitrogen stable isotope ratios to determine the sources of nutrients and contaminants in surface water and groundwater is a regular practice, especially when the nitrogen species come from single or well identified sources, such as commercial fertilizers, sewage systems or treatment plants (Figure 5.2; Komor and Anderson Jr 1993; Aravena and Robertson 1998; Lake et al. 2001; Miyajima et al 2009). When multiple sources are involved, the interpretation of the stable isotopes analyses may not be straightforward, due to the transformations causing differential isotopic fractionation or mixing of substrates and products (Hood *et al*. 2014; Mayer *et al*. 2013).

The preferential use of the ^{14}N isotope over the ^{15}N isotope has been reported for algae and macrophytes (Needoba et al. 2003, Jones et al. 2004). This preference is assumed to be the result of the enzymatic expression, temperature, physiological stress and substrate saturation (Mariotti et al., 1982; Handley and Raven, 1992). Discrimination against the heavy isotope has been quantified experimentally for the enzymes nitrate reductase and glutamine synthetase (+15 to +16‰, Pritchard and Guy, 2005). Therefore, as biological reactions proceed, the discrimination against ^{15}N is evident in the substrate; thus, the pool of available nitrogen becomes enriched in the heavy isotope (Cernusak et al. 2009).

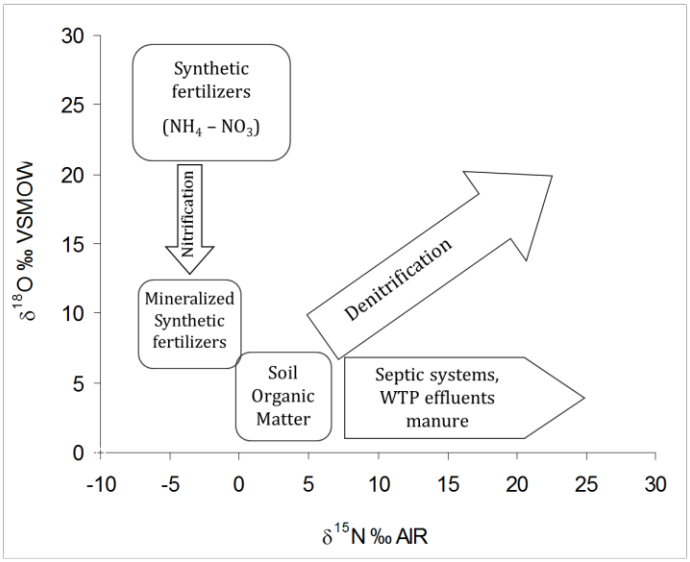


Figure 5.2- Isotopic composition of nitrate ($\delta^{15}N_{NO_3^-}$) of different substrates and the processes leading to changes in the isotopic composition of the products. Modified from Clark and Aravena (2005)

The main processes changing the isotope delta of ammonium and nitrate in aquatic ecosystems are ammonia volatilization, biological uptake, ammonium oxidation and denitrification. Each process has a range of enrichment factors (Table 5.1). The enrichment factor is the difference between the nitrogen isotopic composition ($\delta^{15}N$) of the substrate and product expressed as per mill (‰).

Table 5.1 - Isotope fractionation ε (expressed in ‰) observed as a result of the most common nitrogen-involved processes. Data taken from [1]Högberg (1997), [2]Robinson (2001), [3]Sebilo et al. (2002) and [4]Evans. (2007).

PROCESS	ε ^{15}N/‰
[2] NH_3 volatilization	40 - 60
[1,2] NH_4^+ assimilation	9 - 18
[1,2] NH_4^+ mineralization	0 – 5
[1,2] N_2 fixation	0 – 6
[2] NO_3^- assimilation	0 - 19
[1,4] Nitrification	15 - 35
[1,3] Denitrification	0-18; <30
[4] NO_3^- immobilization (*microbial*)	13
[4] NH_4^+ immobilization (*microbial*)	14-20

The processes occurring in the river (*e.g*. nitrification, uptake) and in the soil-water interface modify the isotopic composition of the nitrate and ammonium available for plant uptake and microbial immobilization in the river. Thus, the assimilation of either N species into biomass is likely to reflect the preferential uptake of one species over the other and the potential isotopic fractionation of each species during the assimilation process. Hood *et al*. (2013) found that a large percentage of the macrophyte tissue downstream of a wastewater treatment plant could be attributed to ammonium (86 to 95%), thus suggesting that ammonium was the preferred nitrogen source over nitrate. Similarly, epilithon was found to assimilate preferably ammonium over nitrate (Chapter 2 of this thesis). In terms of the isotope delta, the δ^{15}N of submerged macrophytes that received nutrients from agricultural runoff has been found similar to the dissolved inorganic nitrogen derived from fertilizers (Kohl et al. 1971). Fry (1971) reported algae δ^{15}N from -2 to +3‰ in North America temperate lakes, values similar to the nitrogen isotope delta of the soil organic matter.

The changes in the δ^{15}N of primary producers and consumers in aquatic ecosystems have been used to estimate changes in nutrient sources and the metabolic status of rivers and streams. Fry (1991) found that δ^{15}N close to 0‰ (particularly in seston) suggested N-fixation by legumes and alder close to the water channel; whereas algae were constrained to nitrogen isotope delta between -2 and +3‰, a common δ^{15}N in soil organic matter and synthetic fertilizers. Anderson and Cabana (2006) found that streams impacted by point-source discharges showed changes in the δ^{15}N at three different trophic levels (primary consumers, predatory invertebrates and invertebrate-feeding fish). According to Finlay and Kendall (2007), ^{15}N-enrichment is usually large in human-impacted rivers as a result of the external input of nutrients and the relatively high bioavailability of the dissolved nitrogen species.

In 2013, the Region of Waterloo completed major upgrades to the Kitchener Wastewater Treatment Plant (henceforth referred as KTP), which is a conventional activated sludge process plant with chemical phosphorus removal and sodium hypochlorite disinfection and treatment capacity of 123 million litres per day (MLD, Region of Waterloo 2013). Before upgrades, the effluent was released with high ammonium concentrations. After upgrades, better aeration led to almost complete nitrification (Region of Waterloo 2014). Before upgrades, the effluent

released with high ammonium concentration was oxidized in the river, and the $\delta^{15}N_{NH_4^+}$ showed ^{15}N-enrichment with distance. After upgrades, almost complete nitrification inside the KTP resulted in elevated nitrate concentrations and a small pool of ^{15}N-enriched ammonium in the effluent.

To my knowledge, there are few studies describing the changes in the $\delta^{15}N$ of the aquatic community as a result of changes in the quality of the water body receiving discharges from a wastewater treatment plant. The objective of this chapter is to provide evidence of the helpfulness of periphyton and macrophytes as a short-term environmental archive, capturing the effect of the changes in the effluent of the Kitchener wastewater treatment plant upon the biomass isotopic composition. It was hypothesized that the isotopic composition of seston, periphyton and macrophytes at different distance from the KTP efluent would be similar to the $\delta^{15}N$ of the ammonium at that location ($\delta^{15}N_{NH_4^+}$), assuming that NH_4^+ is incorporated without energy consumption and preferred over NO_3^- (Dortch 1990).

MATERIALS AND METHODS

Study Area

The samples used to assess the isotope delta of seston, macrophytes and periphyton ($\delta^{15}N_{TN}$ and $\delta^{13}C_{TC}$) were collected at different locations upstream and downstream of the Kitchener Wastewater Treatment Plant (KTP, Figure 3), spanning a total of 5.7 km downstream of the KTP. The upstream location (Victoria, 14 km upstream of the KTP effluent) represents the agricultural sub-catchments and the Waterloo wastewater treatment plant. The downstream locations collect the impacts of the KTP effluent at different distances from the discharge, with different NH_4^+ and NO_3^- concentrations and isotopic compositions.

Sample collection

The KTP underwent a series of upgrades that were completed in January 2013. Submersed macrophytes, periphyton (epilithon and epiphyton) and seston samples were collected before upgrades (August 2011) and after upgrades (August 2013) at six locations: one upstream (Victoria St., 14 km above the KTP effluent) and five locations downstream of the KTP (Figure 5.3). The samples representing the location at 0 m downstream estimates the concentration

obtained due to instantaneous volumetric mixing of effluent to the river discharge. All biomass samples downstream of the KTP effluent were collected inside the plume of nutrients created by the KTP effluent (as described in Chapter 4).

Samples for water quality analysis were collected biweekly or monthly in HDPE containers, filtered to 0.45 µm (Whatman membrane filter) and stored cold or frozen before analysis. Water samples for isotopic analyses of this reach of the Grand River were occasionally analysed from 2007 to 2013 in different seasons, in order to capture the variability of the $\delta^{15}N$ of ammonium and nitrate observed in the Central Grand River.

Code	Location	Distance from WTP (m)
PT	Pioneer Tower	520
OMR	Old Mill Rd.	880
GC	Doon Valley Golf Club	2600
FL	Floods (Edgehill St.)	3500
BL	Blair	5700

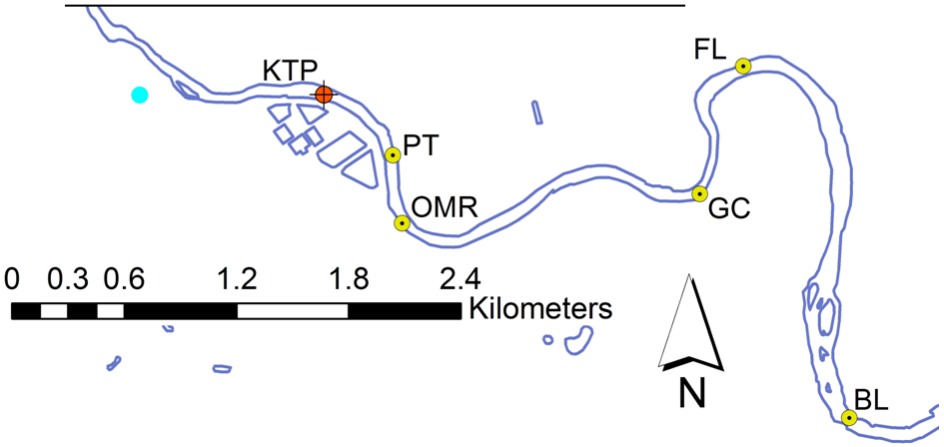


Figure 5.3 - Sampling locations at the Central Grand River downstream of the Kitchener Wastewater Treatment Plant. Samples were collected before (2011) and after (2013) upgrades. The upstream location is out of the map (Victoria St., 14 km upstream of the KTP effluent)

Macrophytes, periphyton (epiphyton and epilithon) and seston samples were collected as follows:

· *Macrophytes*. Two samples of the whole organism were collected at each location, including above and below ground biomass, avoiding ruptures of breakage. The species collected were *Myriophyllum spicatum, Elodea sp*. *Fontinalis* and *Potamogeton* spp (which

could include species of the genus *Potamogeton* and *Stuckenia pectinata*). Mats of *Cladophora* were treated as macrophytes. The material was slightly rinsed *in situ* to eliminate excess sediments and superficial debris; immediately stored in plastic bags (Ziploc ®) with sufficient water to preserve them wet and then stored on ice. Back in the laboratory, each macrophyte (stem, roots, leaves and reproductive structures if present) was placed in a mason jar (1 1itre) with approximately 50 ml of nanopure water and vigorously agitated. The slurry, comprising all detached material, was considered epiphyton (see below). Each macrophyte was then submitted to extensive washing (distilled water), then blotted and oven dried in paper bags (60ºC, 48-72 hrs). The dry material of each macrophyte was pulverized (Retsch Mixer Mill MM200), acid-washed (5% v/v HCl) to remove carbonates, oven dried and stored in acid-washed glass vials until elemental analysis.

· *Epiphyton*. The slurry obtained from each macrophyte (n=2) was decanted into centrifuge tubes, frozen and freeze-dried. Sub-samples were acidified (5% v/v HCl), oven dried and stored in acid-washed glass vials until elemental analysis.

· *Epilithon*. Two cobbles (≈10 cm diameter) at each location were collected directly from the river and stored in plastic bags (Ziploc ®) with sufficient water to preserve them wet, and then stored on ice. In the laboratory, all material covering the rock was obtained by scraping (stainless steel spatula) and brushing (soft toothbrush) the surface of the cobbles. Approximately 50 ml of nanopure water was used to obtain all biomass (plus sediments). The slurry was decanted to remove coarse mineral material and centrifuged to concentrate it. The resulting pellet was frozen and freeze-dried, acidified (5% v/v HCl), oven dried and stored in acid-washed glass vials until elemental analysis. Occasionally, sub-samples of supernatant from previous centrifuge step that did not precipitate at 2000 rpm were filtered (quartz filter, 1.2 μm pore size) for analysis of macrogels and colloidal material grouped as external polymeric substance (EPS), which includes high-molecular-weight mixture of polymers and possibly picoplankton (0.2-2.0 μm) that did not pellet as organic matter during centrifugation (OECD Guidelines for testing chemical 2009).

· *Seston*. An open cylinder (10 cm diameter) holding a 70μm mesh was submerged at 15 cm depth in the Grand River, and allowed to collect the floating material for one minute or until suspended solids were evident on the mesh (single sample). The material was transferred to a 20 ml HDPE container, stored in ice. Back in the laboratory, samples were frozen and freeze-dried, acidified (5% v/v HCl), oven dried and stored in acid-washed glass vials until elemental analysis.

Analyses of samples

Water

NH_4^+ and NO_2^- were measured by colorimetric methods (blue indophenol and with sulphanilamide + azo dye respectively) using a Beckman UV spectrophotometer and Smartchem 200 Autoanalyzer (precision 5%, detection limit 0.05 mgN/L). Ammonium samples were acidified prior to analysis, thus NH_4^+ represent NH_3+NH_4^+ henceforth. NO_3^- was measured with an Ion Chromatograph (Dionex ICS-90; precision 0.07 mg N/L, detection limit 0.05 mg N/L).

The isotopic samples for nitrate after the KTP upgrades were analysed in the Stable Isotope Facility (SIF), University California, Davis. The rest of the isotopic samples (nitrate before upgrades, ammonium, seston, periphyton and macrophytes) were analysed in the Environmental Isotope Laboratory (EIL), University of Waterloo. $\delta^{15}N_{NO_3^-}$ was obtained by chemical denitrification (NO_3 to N_2O) as described by McIlvin and Altabet (2005). $\delta^{15}N_{NH_4^+}$ was obtained by an alkaline diffusion following Spoelstra *et al*. (2006). Isotopic analyses of nitrate and ammonia were done in duplicate using a GV Trace Gas preconcentrator systems coupled to a GVIsoprime mass spectrometer. Stable isotope ratios are expressed as delta (δ) and are expressed per mil (‰) relative to atmospheric air with an analytical precision of ±0.3‰ for ^{15}N. Standards were prepared from IAEA 311 ammonium sulphate salts, characterized by the Environmental Isotopes Laboratory – University of Waterloo using IAEA-N-1 (+0.43‰), IAEA-N-2 (+20.41‰) and IAES 305B (+39.8‰).

A total of 59 samples of $\delta^{15}N_{NH_4^+}$ and 36 samples of $\delta^{15}N_{NO_3^-}$ were included in the analysis, in order to represent the before upgrades conditions at different seasons from 2007 to 2012. This

approach allowed the incorporation of an ample database into the *most probable region* polygon of $\delta^{15}N_{NO_3^-}$ (+1 to +11.4 ‰) and $\delta^{15}N_{NH_4^+}$ (+2.5 to +30 ‰) nitrogen isotope delta before the KTP upgrades. The polygons were drawn to include all the points in a scatter plot and comprise the intra- and inter-annual variability in the nitrogen isotope delta of each species observed in the Central Grand River downstream of the KTP before the upgrades. The *most probable region* polygons for the $\delta^{15}N_{NH_4^+}$ (+23 to +30.8 ‰) and the $\delta^{15}N_{NO_3^-}$ (+10.5 to +14.2 ‰) after the KTP upgrades were delineated using samples from 2013 only.

$\delta^{13}C_{DIC}$ was obtained in duplicate from equilibrated headspace (He) after acidification (H_3PO_4 85%) to convert all dissolved inorganic carbon into CO_2. The gas was analysed by Isochrome continuous flow GC-C-IRMS (Isotope Ratio Mass Spectrometer) and results referred to NBS19 ($\delta^{13}C$=1.95‰). $\delta^{13}C_{DOC}$ samples were stripped of CO_2 and submitted to anaerobic oxidation of organic carbon (potassium persulfate) and analysed on an Isoprime Mass Spectrometer. Stable isotope ratios are expressed as delta (δ) and are expressed in per mill (‰) relative to the carbonate rock Peedee Belemnite with an analytical precision of ±0.2‰ for ^{13}C.

Biomass isotopes analyses

Oven dried biomass samples (duplicate) were analysed with a Delta Plus, Continuous Flow Stable Isotope Ratio Mass Spectrometer (Thermo Finnigan Bremen-Germany) coupled to a Carlo Erba Elemental Analyzer (CHNS-O EA1108 - Italy). Stable isotope ratios are expressed as delta (δ) and are expressed in per mil (‰) relative to atmospheric nitrogen and the carbonate rock Peedee Belemnite for carbon. Analytical error for clean, ball-milled material was ± 0.3‰ for nitrogen and ±0.2‰ for carbon. Nitrogen and Carbon compositions were calculated based on Carlo Erba Elemental Standards B2005, B2035 and B2036 with an error of ±1%.

The external polymeric substance (EPS) is considered here as the material collected on 1.2 µm quartz filters. The filters (quartz filters) were selected considering the instrumentation used for the analysis; however, the pore size could have retained suspended solids and cells that did not pellet by centrifugation.

Paired T-test were done for comparing the isotope delta of each biotic component at the same location before and after the KTP upgrades (t-test, significance value α=0.05) using the software JMP 5.1. Some graphics were done in SPSS 13.

RESULTS

Changes in the ammonium and nitrate concentrations in the KTP effluent

Before upgrades (2010-2012), the KTP released an effluent highly concentrated in ammonium (22-36 mgN-NH_4^+/L, Figure 5.4). An important part of that ammonium was nitrified in the Grand River in the 5700 metres below the effluent discharge (approximately 30%, see Chapter 6), impacting the dissolved oxygen particularly during summer, low-flow conditions. Nitrate in the KTP effluent before upgrades was between 1.2 and 4.9 mgN-NO_3^-/L. BOD5 varied between 5 and 13 mg/L and TP had an average 0.6 mg P/L (± 0.21) in July of 2010 and 2011 (Region of Waterloo 2014). After the KTP upgrades, the effluent had approximately 24 mgN-NO_3^-/L, with ammonium concentrations below 4 mgN-NH_4^+/L (monthly median, Figure 5.4). Total phosphorus oscillated between 0.16 and 1.0 mg/L (monthly median); whereas BOD5 varied from ≈5 to 8 mg/L (monthly median) after upgrades. No information regarding dissolved organic carbon was available (Region of Waterloo 2014).

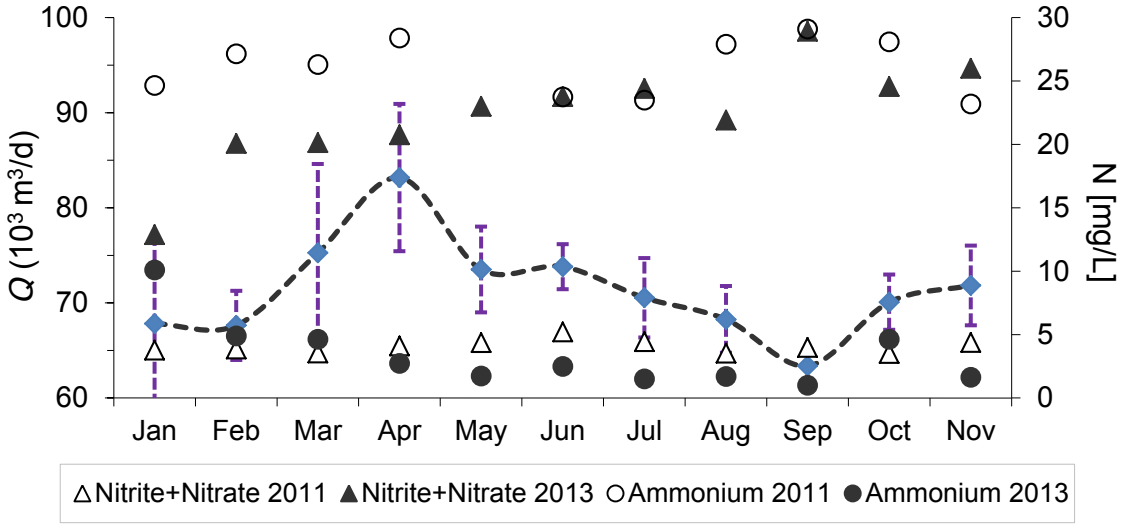


Figure 5.4 - Ammonium and nitrite+nitrate in the effluent of the Kitchener wastewater treatment plant before (2011) and after (2013) upgrades; monthly median (Region of Waterloo 2014). The dashed line represents monthly average (± s.d.) of the volumetric discharge from the Kitchener wastewater treatment plant in 2013 (Q in 10^3 m^3/d). Data provided by the Region of Waterloo.

Changes in NH_4^+ and NO_3^- concentrations and isotopic composition in the Grand River downstream of the KTP effluent

As a result of the upgrades in the operation of the KTP, the ammonium and nitrate concentrations in the Grand River downstream of the KTP effluent changed, decreasing the former and increasing the latter. In the summer of the year 2011 (low flow conditions before upgrades), the ammonium concentration downstream of the KTP effluent through the day decreased from around 4 mg N-NH_4^+/L at 1 m below the discharge (estimated by volumetric dilution of the KTP effluent discharged into the Grand River) to 0.21 N-NH_4^+/L (±0.2) at 5700 m downstream of the KTP (Figure 5.5). In the summer of 2013, after the KTP upgrades, most of the ammonium was nitrified inside the KTP, thus the ammonium in the Grand River 5700 m downstream of the KTP was reduced to ≤ 0.4 mg N-NH_4^+/L (Figure 5.5).

The nitrate concentration in the Grand River downstream of the KTP in 2011 (before the upgrades) increased in average 0.7 mg N-NO_3^-/L between the effluent and the location 5700 m (see chapter 4 for details). However, in the summer after the KTP upgrades (2013), the reach of the Grand River close to the KTP effluent had an increase in the nitrate concentration of almost 2 mg N-NO_3^-/L compared to upstream conditions, and was as high as 3.3 mg N-NO_3^-/L at the location 5700 m downstream the KTP (solid triangles in Figure 5.5).

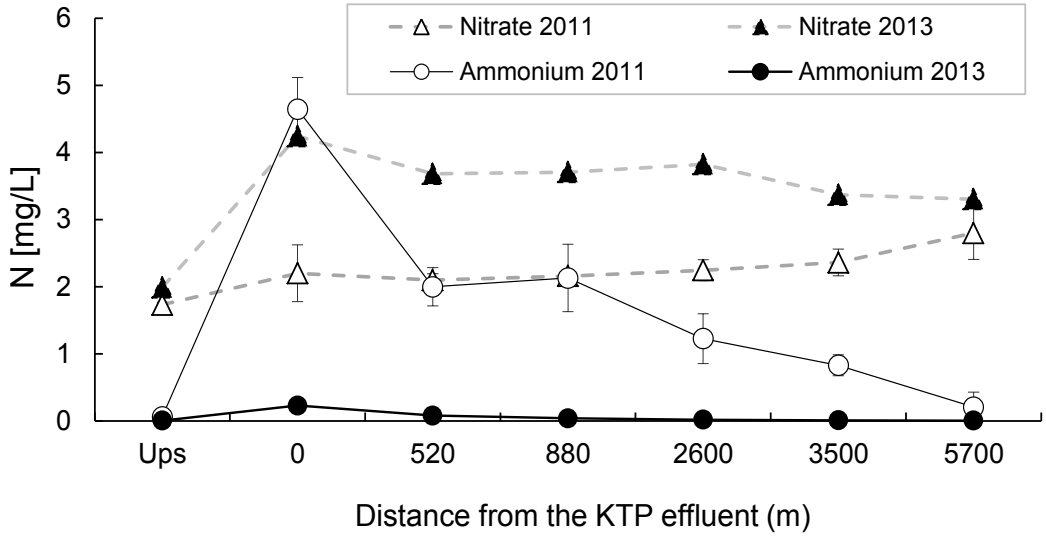


Figure 5.5 - Ammonium and nitrate observed in the Central Grand River downstream of the Kitchener wastewater treatment plant before (2011) and after (2013) upgrades. 2011 represents average ± s.d of five samples collected during one day in summer; one sampling event during the day time in the summer of 2013. Concentrations at 0 m were estimated by volumetric dilution of the KTP effluent discharged into the Grand River. For details about the sampling strategy please refer to Chapter 4.

Year-round, the ammonium concentrations before the KTP upgrades (2011) at the location 5700 m downstream of the KTP were close to 1 mg $N\text{-}NH_4^+$/L during the cold temperature season (fall through winter, Figure 5.6). After the upgrades, the ammonium concentration was reduced to 0.5 mg $N\text{-}NH_4^+$/L or even below detection limit (lower than 0.01 mg $N\text{-}NH_4^+$/L). The nitrate concentrations year-round before and after upgrades had similar seasonal patterns; however, nitrate concentrations in the Grand River downstream of the KTP were higher after upgrades as a result of almost complete nitrification inside the KTP (Figure 5.6, solid triangles). For a detailed description of the annual DIN patterns and concentrations, please refer to Chapter 6 in this thesis.

The ammonium and nitrate isotopic composition ($\delta^{15}N$) observed downstream of the KTP effluent in summer low flow conditions in 2011 and 2013 are shown in the Figure 5.7. These data represent the years when the macrophytes and periphyton samples were collected and have been included as part of the most probable region polygon of the $\delta^{15}N$ of ammonium and nitrate.

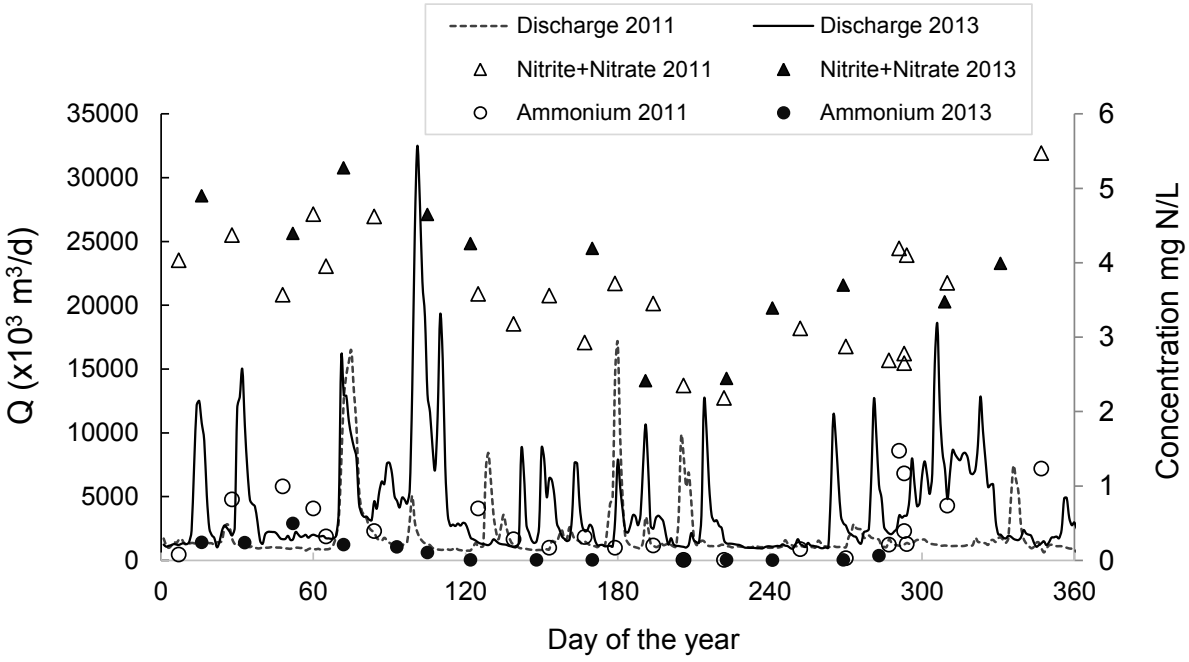


Figure 5.6 - Annual patterns in ammonium and nitrite+nitrate concentrations in the Central Grand River at the location Blair (5700 m downstream of the Kitchener wastewater treatment plant effluent) before (2011) and after (2013) upgrades. The lines represent daily average discharge in the Grand River (*Q* in 10^3 m^3/d) at the station Doon (02GA048, Water Survey of Canada).

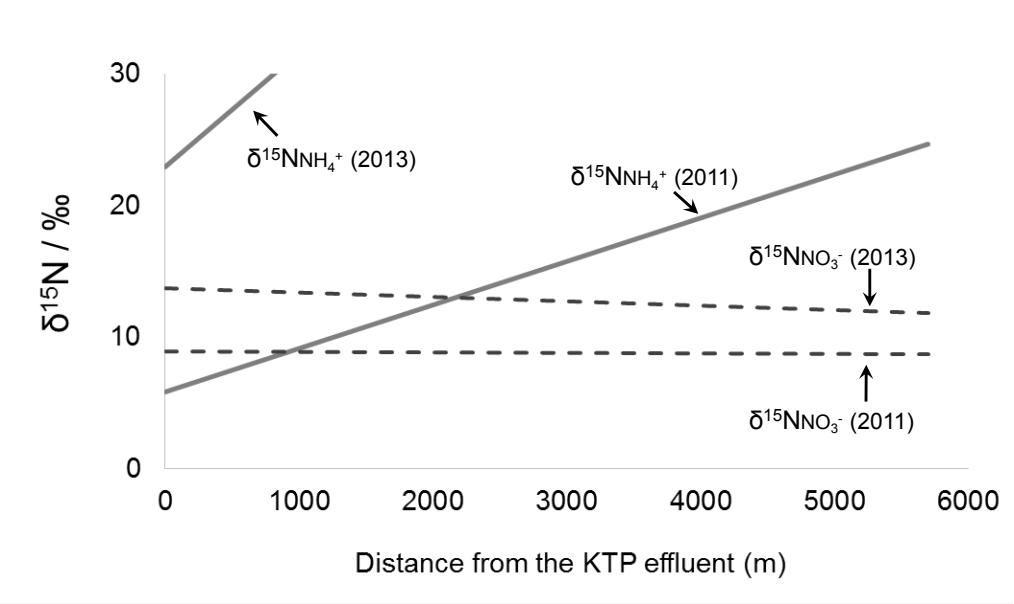


Figure 5.7 - Ammonium ($\delta^{15}N_{NH_4^+}$) and nitrate isotope delta ($\delta^{15}N_{NO_3^-}$) observed in the Central Grand River downstream of the Kitchener wastewater treatment plant before (2011) and after (2013) upgrades. For details about sampling strategy refer to chapter 4 in this thesis.

Overall changes in the $\delta^{15}N$ and $\delta^{13}C$ of macrophytes and periphyton

The samples used for this research aimed to represent the biomass of the current-year growing season (*i.e*, the year where they were collected, 2011 and 2013). Macrophytes and epiphyton are considered to represent the current year, since most (if not all) of the living tissue die during winter. On the other hand, part of the epilithon could have overwintered on top of some cobbles or did not detach completely after decaying; thus, a mixture of dead and live biomass could exist in the samples collected. Seston (the floating part of the periphyton) is moving as the river flows; so, it could represent biomass and detritus moving from the upper sections of the watershed (either urban or agricultural), detached material originated elsewhere upstream relative to the KTP effluent or growth as the river moves through each section.

The samples representing the isotopic composition of the upstream biomass have been included for comparisons and were not included in the statistical analyses. It is important to highlight that the actual upstream sampling location was located 14 km upstream of the KTP effluent, thus the distance axis are not to scale in Figure 5.8.

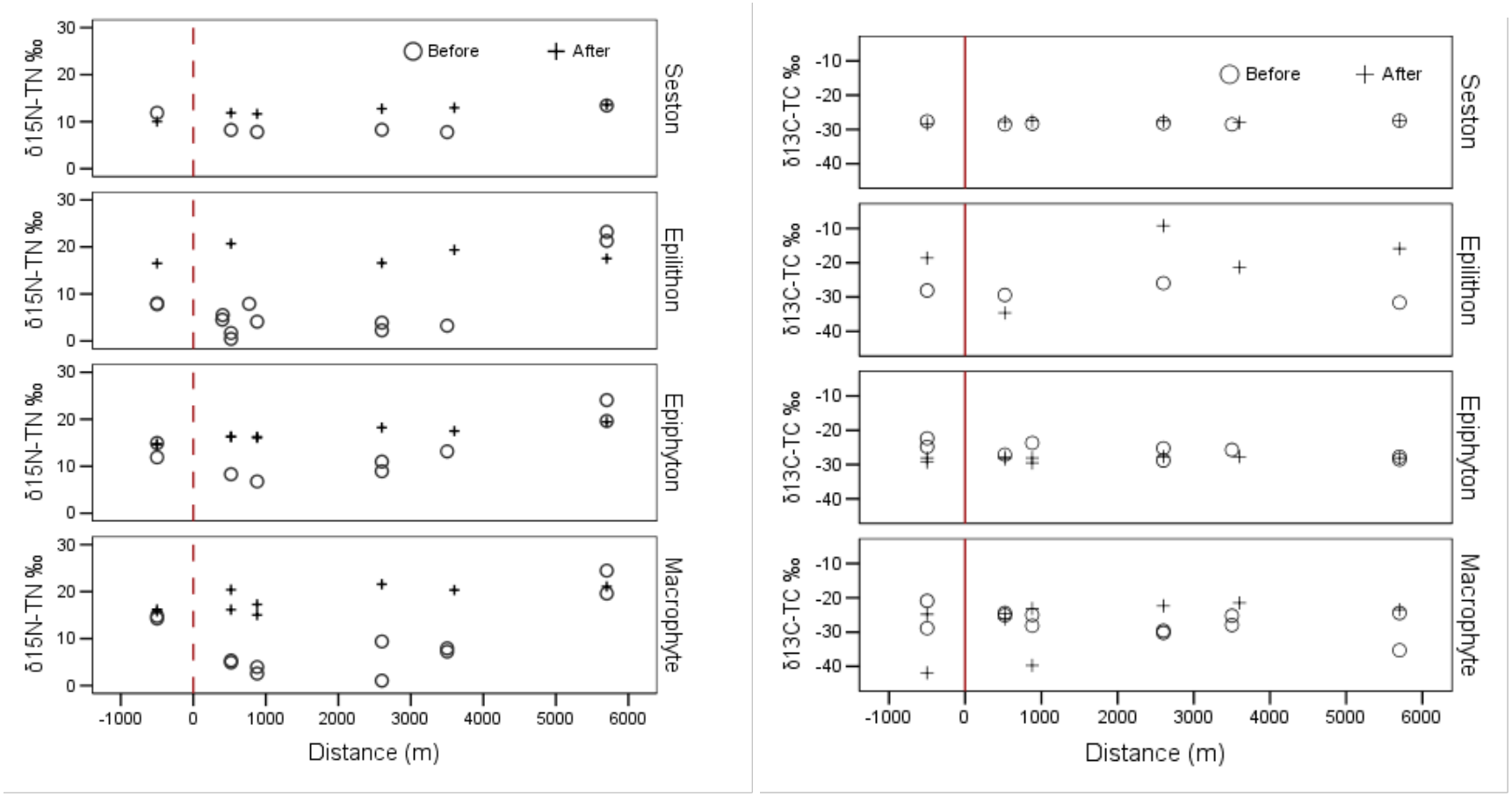


Figure 5.8 - Nitrogen ($\delta^{15}N_{TN}$, left panel) and carbon ($\delta^{13}C_{TC}$, right panel) isotopic composition of macrophytes and periphyton in the Central Grand River. Vertical line represents Kitchener Wastewater treatment plant discharge location (0 m). Data represent before (○ 2011) and after (+ 2013) upgrades. The actual distance of the upstream location is -14000 m.

With some exceptions, the $\delta^{15}N_{TN}$ of most of the biotic samples collected after the KTP upgrades showed a shift equal or greater than 1.5‰ downstream of the KTP effluent, higher after the upgrades. There were significant differences in the $\delta^{15}N$ of epilithon (t=-11.35, p<0.001) macrophytes (t= -8.57, p=0.0018) and seston (t=-2.56, p<0.05) when comparing samples downstream of the KTP before and after the upgrades (Figure 5.8, *left*). No statistical differences were observed in the epiphyton isotopic composition downstream of the KTP comparing before and after the KTP upgrades.

Changes in the carbon isotope delta were not expected as a result of the KTP upgrades, given that the operation upgrades were aimed to nitrogen only. However, the epilithon $\delta^{13}C_{TC}$ was significantly different before and after upgrades (t=2.96, p=0.0091; Figure 5.8, *right*).

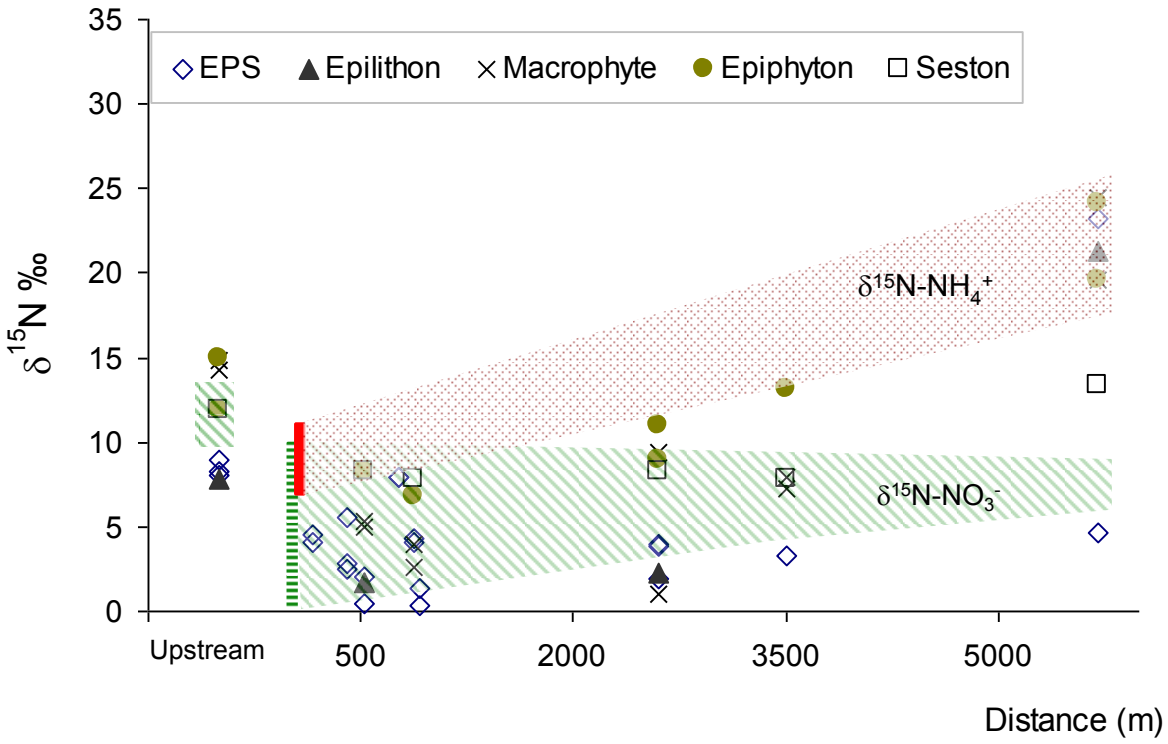


Figure 5.9 - Nitrogen isotope delta of macrophytes, periphyton, seston and the extra polymeric material (EPS) before Kitchener wastewater treatment plant upgrades (August 2011).The *upstream* samples were collected 14 km above of the KTP effluent. The shaded polygons represent the most probable region of $\delta^{15}N_{NH_4^+}$ (*grid*) and $\delta^{15}N_{NO_3^-}$ (*diagonals*) before upgrades (2007 to 2012). Rectangles at 0 m represent the Kitchener wastewater treatment plant discharge and $\delta^{15}N$ interval for the same period. Analytical error ±0.3‰ for ^{15}N.

Changes in the $\delta^{15}N$ in the Central Grand River before and after the KTP upgrades

Before upgrades, the $\delta^{15}N_{NO_3^-}$ upstream (-14000 m) was between +8 and +12‰; whereas biomass $\delta^{15}N_{TN}$ fell between +8 and +15‰ (Figure 5.9). The ammonium concentration upstream of the KTP was below the concentration limit for the isotopic composition analysis procedure as described in *analyses of samples* (see above *Materials and methods*).

Before upgrades, the $\delta^{15}N_{NO_3^-}$ in the KTP effluent was above +1‰ and the $\delta^{15}N_{NH_4^+}$ was between +4.4 and +9.5‰ (rectangles at 0 m, Figure 5.9). No biological samples were collected in the KTP effluent.

Before upgrades in the KTP, the $\delta^{15}N_{NH_4^+}$ in the Grand River downstream of the KTP effluent followed a pattern in ^{15}N-enrichment to $\delta^{15}N_{NH_4^+}$ as high as +30‰. This increase in the $\delta^{15}N_{NH_4^+}$ is assumed to be caused by ammonia volatilization, ammonium oxidation and assimilation (refer to Chapter 4 for more details). The $\delta^{15}N_{NO_3^-}$ was from +6.5 to +10 ‰.

The $\delta^{15}N$ of some seston, epiphyton and epilithon samples downstream of the KTP effluent before the KTP upgrades, increased with distance (from 520 to 5700 m); whereas the macrophyte $\delta^{15}N$ was greatly variable (Table 5.2). Most of the EPS samples had similar $\delta^{15}N$ to the $\delta^{15}N_{NO_3^-}$ along the sampled reach of the Grand River, with the exception of a sample at 5700 m (+23.2‰, Figure 5.9).

After upgrades (2013) the $\delta^{15}N_{NO_3^-}$ upstream of the KTP was not different than before the KTP upgrades (between 10 and 12 ‰). The $\delta^{15}N$ of macrophytes and periphyton at the location upstream of the KTP was close to 15‰, showing less variability than in 2011. The samples representing the upstream location were collected downstream of the Waterloo WTP; however there were not changes directly attributed to the Waterloo WTP effluent. The ammonium concentration upstream of the KTP after upgrades was also below the concentration limit for isotopic composition analysis.

Table 5.2 - Nitrogen ($\delta^{15}N_{TN}$) and carbon ($\delta^{13}C_{TC}$) isotope delta of macrophytes before (2011) and after (2013) upgrades in the Kitchener wastewater treatment plant at different location from the effluent discharge. *Ups* location is 14 km upstream of the effluent. *Stuckenia pectinata* and species of the genus *Potamogeton* have been grouped as *Potamogeton* spp. All samples were acid-washed before the isotopes analysis.

Year	Distance from the KTP (m)	Species	$\delta^{15}N_{TN}$	$\delta^{13}C_{TC}$
2011	Ups	*Myriophyllum spicatum* and *Potamogeton* spp.	14.8	-20.9
		Cladophora	14.3	-28.9
	520	*Potamogeton* spp.	5.3	-24.5
		Myriophyllum spicatum	4.9	-25.1
	880	*Myriophyllum spicatum*	3.9	-25.0
		Cladophora	2.6	-28.2
	2600	*Cladophora*	1.1	-30.2
		Potamogeton spp.	9.4	-29.6
	3500	*Myriophyllum spicatum*	7.9	-27.9
		Potamogeton spp.	7.2	-25.2
	5700	*Myriophyllum spicatum* and *Potamogeton* spp.	19.6	-24.4
		Elodea canadensis	24.5	-35.3
2013	Ups	Myriophyllum spicatum	15.8	-24.8
		Fontinalis sp.	16.2	-41.9
	520	*Myriophyllum spicatum*	20.4	-26.2
		Potamogeton spp.	16.1	-24.5
	880	*Potamogeton* spp.	17.3	-23.1
		Fontinalis sp.	15.0	-39.7
	2600	*Potamogeton* spp.	21.6	-22.4
	3600	*Potamogeton* spp.	20.3	-21.5
	5700	*Potamogeton* spp.	21.1	-23.4

The KTP effluent after the upgrades had a $\delta^{15}N_{NH_4^+}$ from +23 to +31‰ as a result of almost complete ammonium oxidation inside the KTP (Figure 5.10). The $\delta^{15}N_{NO_3^-}$ in the effluent was higher than before upgrades, between +12.8 and +14.2‰ (Figure 5.10) with higher nitrate concentration (22 mg $N\text{-}NO_3^-$/L ±).

The $\delta^{15}N_{NO_3^-}$ in the Grand River downstream of the KTP effluent after the upgrades was between +10.5 and +13.8‰. The $\delta^{15}N_{NH_4^+}$ close to the KTP effluent was 26 and 30‰ (520 and 880 m downstream of the KTP; Figure 5.10). Due to analytical limitations, samples with

ammonium concentrations lower that 0.5 mg $N\text{-}NH_4^+$/L (further than 1 km downstream of the KTP effluent) were not analyzed for isotopes; however, an incresing trend in the $\delta^{15}N_{NH_4^+}$ is suggested by the two samples analysed.

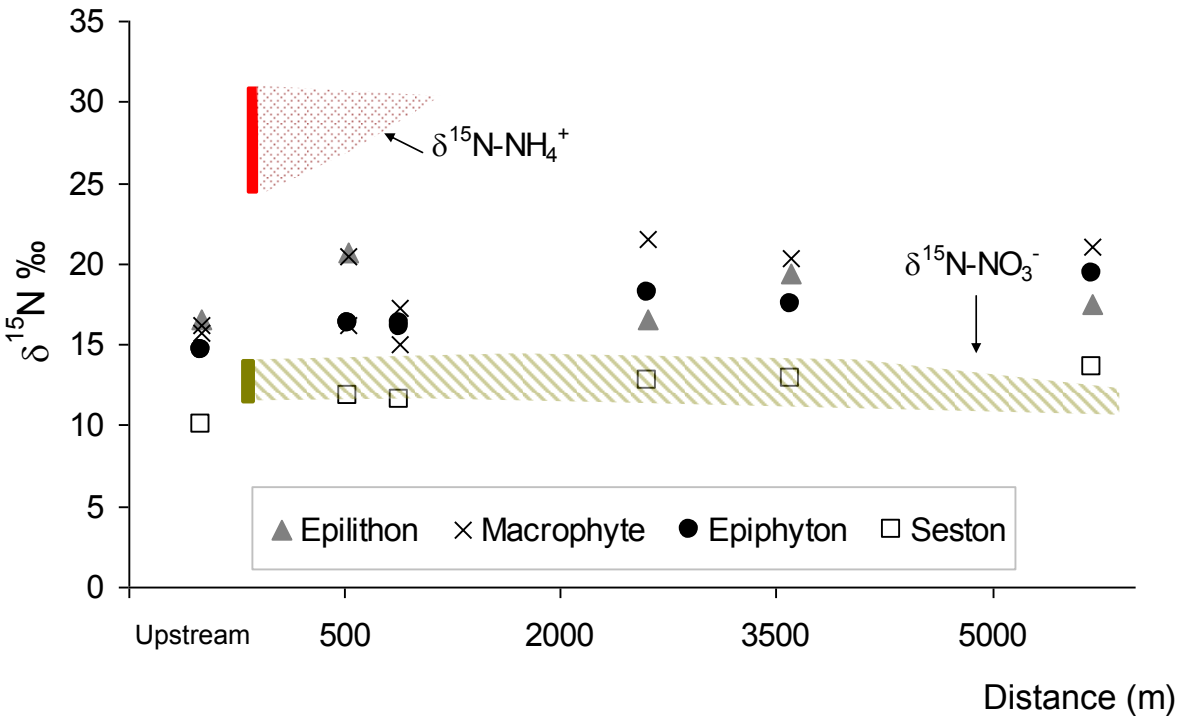


Figure 5.10 - Nitrogen isotope delta of macrophytes, periphyton and seston after Kitchener wastewater treatment plant upgrades (August 2013).The *upstream* samples were collected 14 km above of the KTP effluent. The shaded polygons represent $\delta^{15}N_{NH_4^+}$ (*grid*) and $\delta^{15}N_{NO_3^-}$ (*diagonals*) observed in 2013. Rectangles at 0 m represent the Kitchener wastewater treatment plant discharge and $\delta^{15}N$ interval for the same period. Analytical error ±0.3‰ for ^{15}N.

In general, the $\delta^{15}N$ of biological samples collected after the KTP upgrades were higher after upgrades than before upgrades. The largest variability in $\delta^{15}N$ was observed in macrophytes (from +14.7 to +21.5‰), whereas seston remained close to +12‰ (Figure 5.10). A summary of the isotopic composition of biomass, ammonium and nitrate is shown in Table 5.3. Due to the fact that the $\delta^{15}N_{TN}$ of some samples collected upstream of the KTP effluent after upgrades was similar to some samples downstream of the KTP effluent , it is possible that some of the differences observed in the $\delta^{15}N$ downstream of the KTP are not directly related to the changes in the quality of the effluent, but likely the result of some inter-annual variations and intra-specific differences.

Table 5.3 - Nitrogen ($\delta^{15}N$) and carbon ($\delta^{13}C$) isotope delta of the dissolved species (NH_4^+, NO_3^-, DIC and DOC) and biomass before and after upgrades in the Kitchener wastewater treatment plant. Values (‰) represent minimum-maximum. Biomass includes macrophytes, epilithon, epiphyton and seston. Analytical error ±0.3‰ for ^{15}N and ±0.2‰ for ^{13}C.

	Upstream			***KTP***		***Downstream***		
	NH_4^+	*NO_3^-*	*Biomass*	*NH_4^+*	*NO_3^-*	*NH_4^+*	*NO_3^-*	*Biomass*
$\delta^{15}N$ (‰) Before	*n.a.*	8 - 12	8 - 15	4.4 - 9.5	1	2.5 - 30	6.4 - 11.4	1 - 25
$\delta^{15}N$ (‰) After	*n.a.*	10 - 12	10.5 - 14	23 - 31	12 - 14	26 - 31	1 - 4	10 - 22
	DIC	*DOC*	*Biomass*	*DIC*	*DOC*	*DIC*	*DOC*	*Biomass*
$\delta^{13}C$ (‰) Before	*-10.2*	-27	-26.2 *to* -20.9	-12.2	-25.4	-10.4 *to* -6.9	-26.8	-35.3 *to* -23.7
$\delta^{13}C$ (‰) After	-10.1 *to* -9.8	*n.a*	-41.9 *to* -18.6	*n.a*	*n.a*	-9.8	*n.a*	-39.7 *to* -9.2

Summarizing, with the $\delta^{15}N$ of the samples collected in the central Grand River after upgrades of the KTP, it is proposed that the nitrogen derived from the KTP effluent is the most important source of N for macrophytes and periphyton downstream of the KTP, provided that the isotopic composition of all other end-members (soil organic matter and terrestrial plant litter) does not seem to explain the $\delta^{15}N$ of the macrophytes and periphyton satisfactorily after upgrades (Figure 5.11).

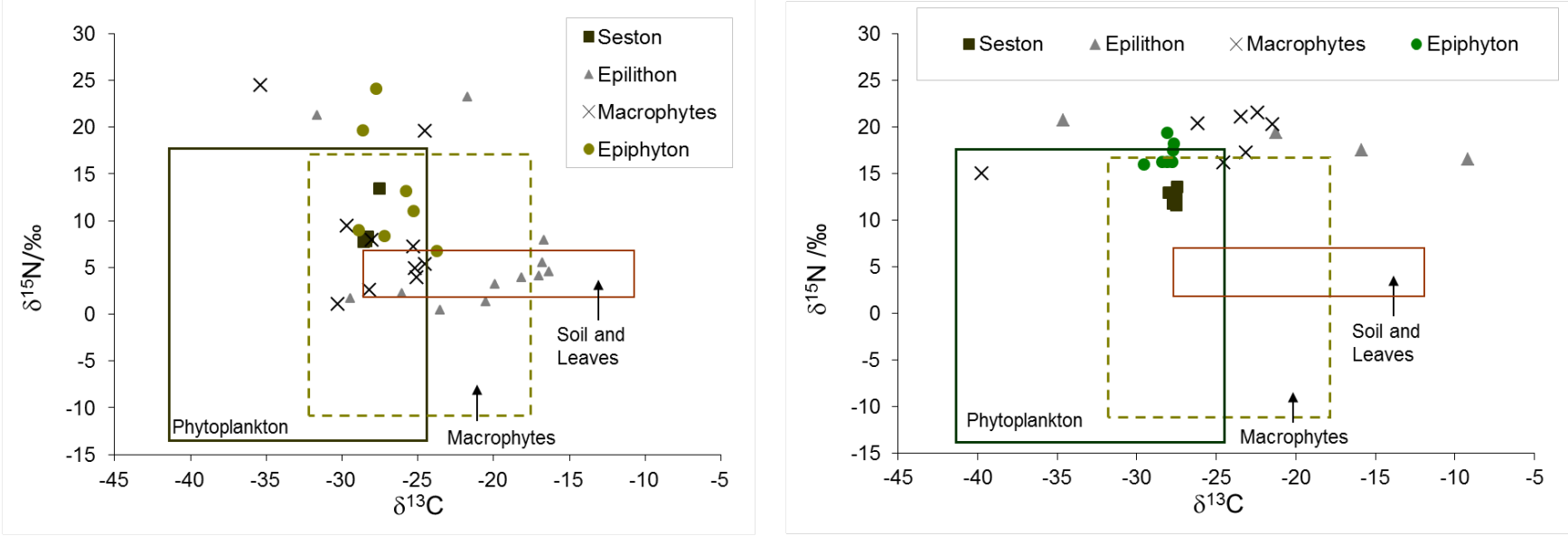


Figure 5.11 - Typical ranges in $\delta^{15}N$ and $\delta^{13}C$ of particulate organic matter in aquatic ecosystems. Samples collected on the Grand River (Ontario) before (2011, *left*) and after (2013, *right*) Kitchener wastewater treatment plantupgrades. Graphs adapted after Finlay and Kendal 2007.

Carbon isotope composition in the central Grand River before and after the KTP upgrades

A limited amount of data collected on the Grand River in 2011 (before upgrades) and 2013 (after upgrades) was used to delineate the most probable region of the dissolved inorganic carbon ($\delta^{13}C_{DIC}$) isotopic composition. The Grand River had a $\delta^{13}C_{DIC}$ around -10‰ (±1‰) and was not different before and after upgrades. The isotope delta of the dissolved organic carbon ($\delta^{13}C_{DOC}$) was only analysed in samples collected in 2011, and it was -26.7‰ (±0.54‰, Figure 5.12). Changes in the $\delta^{13}C$ of macrophytes and periphyton were not expected as result of the KTP upgrades, given that the upgrades targeted reduction in ammonium. Thererofe, the changes in the $\delta^{13}C$ in the aquatic biomass analysed were considered to be a result of inter-annual variations and inter-specific differences among the samples collected.

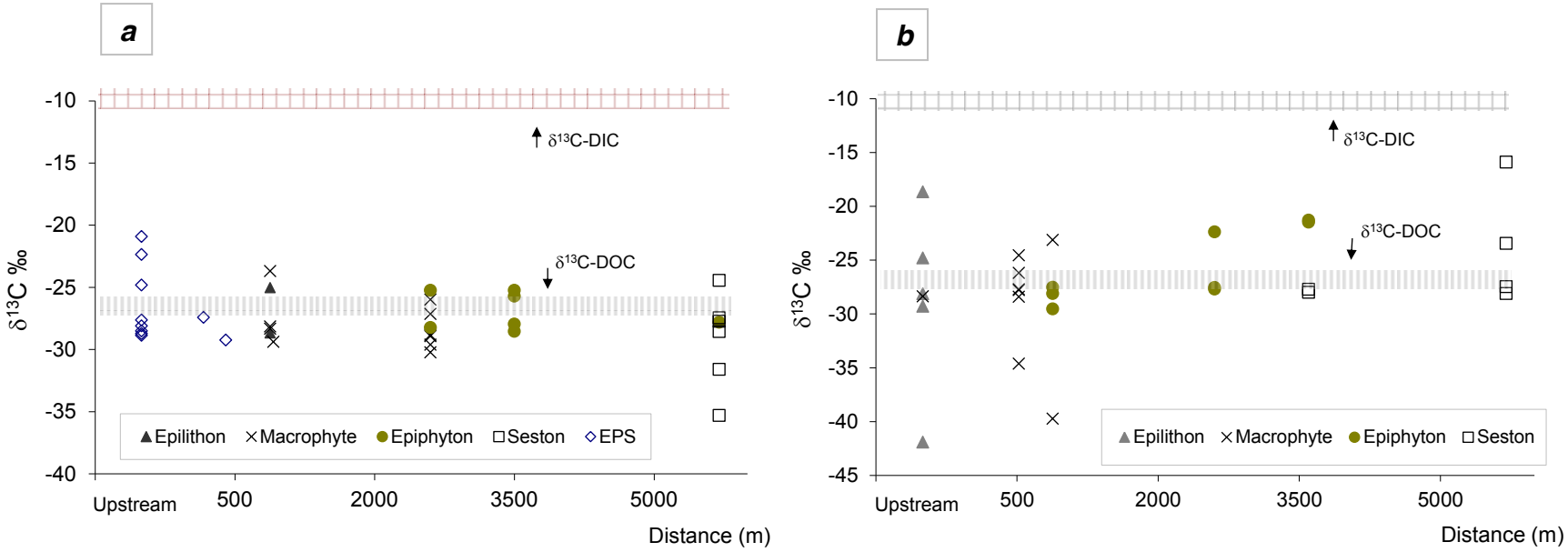
The macrophytes and periphyton samples had variable $\delta^{13}C$, from -30 to -20 ‰, with few macrophyte samples below -35‰ (Figure 5.12*a*). The ^{13}C-depleted value on macrophyte was observed before upgrades in *Elodea sp* (-35‰) at the location 5700 m downstream the KTP and after upgrades in the fountain moss (*Fontinalis sp*) both upstream ($\delta^{13}C$ = - 41.9‰) and downstream of the KTP (-39‰ at the location 880 m downstream the KTP effluent; Figure 5.12*b*).

DISCUSSION

Nitrogen isotope composition and isotopic discrimination in the Grand River

The nitrogen isotope delta of the macrophytes and periphyton was expected to reflect the preferential assimilation of ammonium depending on the similarities between the $\delta^{15}N_{TN}$ of the biological samples and the $\delta^{15}N$ of ammonium; thus, assimilation of nitrate could have provided the observed isotope delta of the submerged biomass.

Figure 5.12**a** (*left*) - Carbon isotope delta of periphyton and seston before Kitchener wastewater treatment plant upgrades (2011). Figure 5.12**b** (*right*) Carbon isotope delta of periphyton and seston after Kitchener wastewater treatment plant upgrades (2013). The *upstream* samples were collected 14 km above of the KTP effluent. The shaded polygons represent $\delta^{13}C_{DIC}$ (*grid*) observed before (2011) and after (2103) upgrades. $\delta^{13}C_{DOC}$ (*vertical lines*) was measured before upgrades only (2011). Analytical error ± 0.2‰ for ^{13}C.



Before upgrades, nitrate was continuously produced downstream of the KTP as a result of ammonium oxidation, and that nitrate had a $\delta^{15}N_{NO_3^-}$ from +5 to +12‰ within the five kilometres monitored. The $\delta^{15}N$ observed in some EPS, seston and macrophytes samples was similar to the $\delta^{15}N_{NO_3^-}$. The low $\delta^{15}N_{TN}$ of some EPS samples is attributed to the secretion and excretion of complex high molecular weight molecules with more ^{14}N then the cells in the epilithon, or the result of cell lysis and organic matter from wastewater; thus leaving behind the heavy ^{15}N molecules on the attached biomass (Handley and Raven 1992) or imparting the isotope delta of dissolved organic matter washed out from the KTP (Sheng et al. 2010).

After upgrades (2013), some macrophytes and periphyton had $\delta^{15}N$ lower than the $\delta^{15}N$ of the ammonium but higher than the $\delta^{15}N$ of the nitrate (i.e, a $\delta^{15}N_{TN}$ between the isotopic composition of the two nitrogen species). Thus, in both years (2011 and 2013) the $\delta^{15}N_{TN}$ of the biological samples could have been the result of ammonium assimilation with discrimination against $^{15}NH_4^+$, assimilation of nitrate with little or no fractionation or the result of assimilation of both N species, leading to mixing of two pools of N with different $\delta^{15}N$. A simple mixing model that assumes 80% assimilation of ammonium and 20% assimilation of nitrate (based on the difference in experimental uptake rates, see Chapter 2) could explain the observed isotope delta of epiphyton before upgrades (approximately 7.8‰ at 520 m, 13‰ at 3600 m and 23.5‰ at 5700 m). The same mixing model does not explain the macrophytes isotope delta before upgrades; thus, ammonium or nitrate assimilation with discrimination against ^{15}N seems plausible.

After upgrades, the macrophytes that had $\delta^{15}N_{TN}$ +18‰ and the periphyton with $\delta^{15}N_{TN}$ +17‰ could have resulted from 50% assimilation of each N species (nitrate +10‰ and ammonium +23‰). It is also possible that after the upgrades, the NH_4^+ is taken up by active transport whereas before the upgrades was mainly taken up by diffusion of NH_3; thus the isotopic fractionation changed as a result of assimilation patterns (diffusion *vs*. active transport). Finally, after the upgrades, the NO_3^- uptake relative to the supply may be small so changes in the $\delta^{15}N_{NO_3^-}$ were not observed.

Preferential assimilation of NH_4^+ over NO_3^- resulted in increases over distance in the $\delta^{15}N_{TN}$ of the biomass with no changes in the $\delta^{15}N_{NO_3^-}$, as reported by Ribot *et al*. (2012). Given that ammonium is the energetically favourable nitrogen species and its availability before upgrades was high, the ammonium was likely preferentially assimilated by macrophyes and periphyton. Ammonium preference in the Grand River was also suggested by Hood et al. (2014) while studying the mass of NH_4^+ assimilated into macrophyte biomass downstream of wastewater treatment plants.

Others studies in streams and rivers receiving wastewater effluents (Cifuentes *et al*. 1989; Ribot *et al*., 2012) reported $\delta^{15}N_{NH_4^+}$ as high as +55‰ with the $\delta^{15}N$ of the periphyton from 15 to 20‰ lower than the ammonium isotopic composition, and the differences between the isotopic composition of the susbtrate (ammonium) and the product (biomass) has been attributed to discrimination against $\delta^{15}N_{NH_4^+}$.

The difference between the substrate (NH_4^+) and the product (total nitrogen biomass; $\Delta=\delta^{15}N_s - \delta^{15}N_p$) is a relatively simple way to estimate the apparent isotope distribution due to assimilation. Assuming that the isotopic fractionation (ε) is simply the separation between a single substrate (ammonoium) and the product (biomass), the fractionation due to ammonium assimilation in the Grand River before upgrades was between 0 and 15 ‰ and from 5 to 10‰ after upgrades. Similar ammonium isotopic fractionation due to assimilation was reported by Fogel and Cifuentes (1993) in experimental conditions and natural ecosystems. Isotopic fractionation due to ammonium assimilation is considered low (Robinson *et al*. 1998); however, enzymatic discrimination has been reported for the enzyme glutamine synthetase as high as 15‰ (Pritchard and Guy 2005), thus rendering large differences between the isotopic composition of the substrate (glutamate+ammonia) and the product (glutamine). Thus, the separation in 15‰ between ammonium and biomass observed in some epiphyton sample after the KTP upgrades can be attributed to enzymatic discrimination if that process is ocurring, This assumption entails that only enzymatic discrimination was occur ing, without considering the variability in $\delta^{15}N_{TN}$ due to nitrate uptake, external ammonoium availability and the possible differet physiological demands of each species .

Hood *et al*. (2014) found that macrophytes $\delta^{15}N$ could be explained by wastewater-derived ammonium ($\delta^{15}NNH_4^+$) if the slopes of the linear regressions of the substrate ($\delta^{15}NNH_4^+$) and the product ($\delta^{15}N$TN macrophyte) were not significantly different. The *y*-intercept represented the isotopic fractionation (ε) due to assimilation (α_{uptake}, where $\alpha=\varepsilon+1$). The isotopic composition of susbtrate (ammonium) and the product (macrophytes) did not have significantly different slopes before upgrades (t=0.91, p=0.36, df=44,) and after upgrades (t=0.34, p=0.07, df=9). Therefore, the observed isotopic fractionation factor due to ammonium assimilation (uptake fractionation) before the KTP upgrades was α_{uptake}= 1.005 and α_{uptake}=1.012 after the upgrades (Figure 5.13). The same procedure for epiphyton after upgrades provided an estimated ammonium uptake fractionation α_{uptake} = 1.012‰. These estimates assumed the isotopic fractionation solely the difference between the substrate and the product isotopic composition, and do not consider the intra-annual and inter-annual variability. The large difference in the isotopic fractionation factor before and after upgrades could be attributed to larger fractionation after upgrades given that the rate limiting step changed due to the lower-than-before ammonium concentration in the Grand River (*i.e* high fractionation at low concentration).

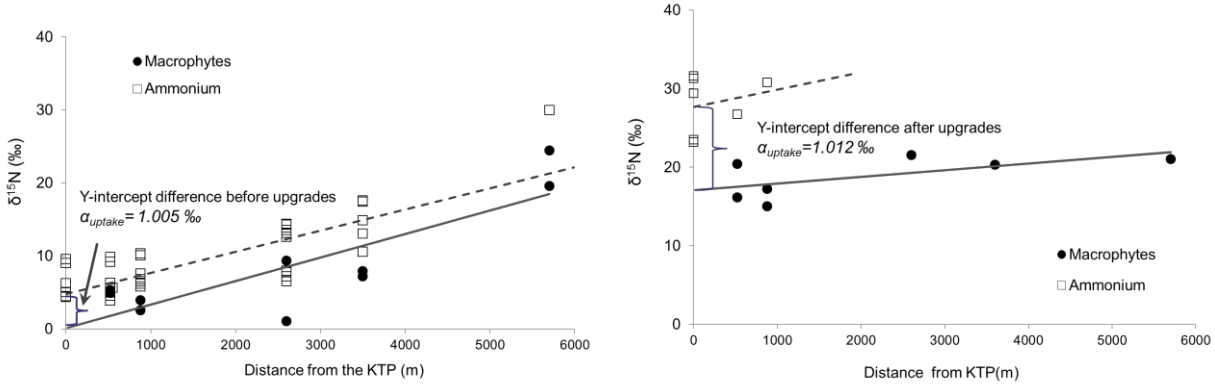


Figure 5.13 - Macrophytes ammonium uptake fractionation factor (α_{uptake}) before (*left*) and after (*right*) upgrades in the Kitchener wastewater treatment plant. Fractionation factor derived from the difference in the *Y* axis intercept between the $\delta^{15}NNH_4^+$ and $\delta^{15}N$TN of the macrophytes when the linear regressions of the substrate and the product are not significantly different.

Another possibility that could contribute to the observed increase in the $\delta^{15}NNH_4^+$ is fast internal ammonium recycling at a micro-scale within the epilithon community, so a very intense ammonium cycling produced a ^{15}N-enriched micro-pool of ammonium around cobbles with dense epilithic mats. The fact that ammonium was not detected by the standard water sampling

methods after upgrades does not imply its complete depletion. Ammonium can remain continuously recycling on a small scale or within the boundaries of the EPS and the area of influence of periphyton. Ribot *et al*. (2012) found that intense heterotrophic activity could trigger high rates of organic matter mineralization; thus regenerating ammonium.

The external N concentration seems to play a role when assessing isotopic fractionation. Some evidence suggests that, when the N supply decreases, discrimination against ^{15}N is reduced or null because the organisms require incorporating nitrogen to fulfill their demands. Therefore, no isotopic fractionation will likely occur, as the N needs to be rapidly incorporated (Andrews, 1986; Robinson, 2001, Cernuzak et al. 2009). On the other hand, nitrogen discrimination at high external concentration is likely to occur due to equilibrium effects ($NH_3 \leftrightarrows NH_4^+$), passive diffusion ($^{14}NH_4^+$ or $^{14}NO_3^-$ will diffuse easily than the molecules with ^{15}N) and enzymatic effects.

Assimilation of nitrate could have also led to some of the changes observed in the $\delta^{15}N$ of some biological samples. Nitrate assimilation by algae has been suggested to be a major control over $\delta^{15}N_{NO_3^-}$, particularly in eutrophic, nitrate-rich rivers (Finlay and Kendall 2007). However such changes in the observed $\delta^{15}N_{NO_3^-}$ in Grand River downstream of the KTP cannot be directly related to nitrate assimilation. The relatively stable $\delta^{15}N_{NO_3^-}$ downstream the KTP could also be explained by the continuous supply of nitrate from the upstream sub-catchments with a relative stable $\delta^{15}N_{NO_3}$ around +11.5‰.

Estimates of isotopic fractionation due to nitrate assimilation into biomass are largely variable. Finlay and Kendall (2007) reported that the $\delta^{15}N$ of algae was 5‰ lower than the $\delta^{15}N_{NO_3^-}$ when nitrate was the only N source; thus discriminating against $^{15}NO_3^-$. Mariotti *et al*. 1984 reported almost no fractionation in estuaries and Fogel and Cifuentes (1993) and Montoya (2007) reported fractionation above 20‰ in experimental conditions and marine environments, respectively.

It is important to highlight that before and after upgrades, the seston $\delta^{15}N_{TN}$ resembled the shift observed in the $\delta^{15}N_{NO_3^-}$, decreasing before upgrades (from 11.8‰ upstream to around 8‰

downstream of the KTP) and increasing after upgrades (from 10‰ upstream to 12.5‰ downstream of the KTP). Thus, the seston could have assimilated nitrate both upstream and downstream the KTP effluent, based only on the similarities in their N isotopic composition. However, given that the seston is comprised by both the living, free-floating fraction (*seston*) and the detritus (*abioseston*) that move with the water, it is very difficult to distinguish between true seston and particulate organic matter upstream and downstream of the KTP and the real origin of their N isotopic composition.

Uptake in *Skeletonema* cell cultures reported $\delta^{15}N_{TN}$ negative fractionation relative to the nitrate in solution (-10 ‰). Ammonium fractionation was measured as -15‰ in cells growing with ammonium at millimolar concentrations, and as negative as -27‰ at micromolar concentrations (Fogel and Cifuentes 1991, Pennock et al 1996). Millimolar concentrations were observed in the Grand River before and after the KTP upgrades (0.01-0.17 mmol $N\text{-}NH_4^+$/L); thus, the -10‰ isotopic separation between the $\delta^{15}N_{NH_4^+}$ and the $\delta^{15}N$ of macrophytes and periphyton is in good agreement the fractionation reported in previous studies. This assumed ammonium isotopic fractionation due to assimilation stands for samples before and after the KTP upgrades as far as 5700 m downstream of the KTP, but it is unknown if it could have been the same at longer distances.

Inter-and intra-specific differences, growth rates and light intensity need to be considered when estimating isotopic fractionation due to assimilation (Montoya and McCarthy 1995). There were changes in the presence of certain species of macrophytes in the Central Grand River downstream of the KTP in the two sampling campaigns (2001 and 2013). For example, *Myriophyllum spicatum* was observed as far as 5700 metres below the KTP effluent before upgrades, whereas after upgrades *Potamogeton* spp dominated most sampled locations. After the KTP upgrades *Cladophora* sp. was not observed in any location. These changes in macrophytes (and algae) species could also be partially responsible for the variability in $\delta^{15}N_{TN}$, as well as promoting changes in periphyton species composition due to changes in the water chemistry and different habitat characteristics. Hood *et al*. (2014) reported different fractionation against ^{15}N by two macrophytes downstream of the KTP; 7.1‰ in *Myriophyllum spicatum* and 2.3‰ for *Potamogeton* spp. So, the average estimated fractionation due to

ammonium assimilation by macrophytes proposed by Hood et al (2014) was 6‰ downstream of the KTP. Such values is in acceptable agreement with the 4.8‰ estimated in this research.

The $\delta^{15}N$ of aquatic biomass in undisturbed rivers has been found to be -1 to +7‰, largely driven by the inputs from terrestrial organic matter (Finlay and Kendall 2007) or representing reduced isotopic fractionation due to low DIN concentrations. The $\delta^{15}N$ of epilithon and particulate organic matter collecetd in the headwaters of the Grand River in 2007 supports this observation (+3.2 to +7.7‰, *unpublished data*). However, this low $\delta^{15}N$ could also be influenced by several factors such as the trophic state of the river in different reaches ($^{15}N_{TN}$ enriched in higher trophic levels), the sources supplying the N to those communities and the human population density (Cabana and Rasmunssen 1996).

Carbon isotope composition and isotopic discrimination in the Grand River

The observed $\delta^{13}C_{DIC}$ in the Grand River upstream and downstream of the KTP was in range common to most rivers, between -15 and -5 ‰ (Σ DIC, Fry and Sheer 1984, Boutton 1991). The $\delta^{13}C_{DOC}$ in the Grand River overlaps with the typical range of terrestrial C_3 plants (-27‰), but also overlaps with the $\delta^{13}C$ of seston and epipyton. Thus, the DOC in the Grand River could be a combination of the organic matter decaying within the river (autochthonous carbon) and in the surrounding lands (allochthonous carbon).

Generally speaking, riverine photosynthetic organisms appear to have a C_3 pathway. Plankton is frequently in the $\delta^{13}C$ range from -30 to -25‰, whereas most aquatic macrophytes are in the range of -30 to -12‰ (Boutton 1991). In the Central Grand River, the $\delta^{13}C$ of the majority of the macrophytes, epilithon and epiphyton was within those ranges, both before and after upgrades; thus, the changes in the observed $\delta^{13}C$ could be attributed to inter-annual and inter-specific variability due to the fact that the upgrades in the KTP aimed to reduce the ammonium in the effluent.

The depletion in ^{13}C of the aquatic biomass could be explained by remineralization of isotopically light CO_2 (*i.e.*, respired CO_2, Fogel and Cifuentes 1993), and changes in the pH (Kendall and Doctor 2003). Exceptionally variable $\delta^{13}C$ for macrophytes in freshwater

ecosystems can occur in the range of 50 to -10‰ (Boutton 1991). High fractionation against ^{13}C is likely due to enzymatic discrimination. Enzymatic discrimination in macrophytes occurs during photosynthesis against ^{13}C by the enzyme Ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO), and has been measured in around 29‰ with respect to dissolved CO_2 (Roeske and O'Leary 1984 and Farquhar *et al*. 1989). Thus, isotopic discrimination of ^{13}C ($\delta^{13}C_{DIC}$= -10‰) resulted in the $\delta^{13}C$ observed on the moss *Fontinalis* sp. upstream of the KTP (-41.9‰) and 880 m downstream of the KTP (-39.7‰).

The Grand River, a river with high primary productivity and important fluctuations in discharge, is expected to have variable $\delta^{13}C$ due to differential biomass development in space and time (Hill and Middleton 2006). In addition to the inter-annual and inter-specific variability, the coexistence of autotrophs and heterotrophs in the epilithon and the epiphyton, could have also contributed to the ample range in the $\delta^{13}C$ observed in the biological samples collected in the Central Grand River. The bulk sample collected as periphyton likely includes algae, bacteria and zooplankton. This complex community has different metabolic pathways, nutrient requirements and nutrients acquisition methods; thus, it is likely to contribute to the variability in the $\delta^{13}C$ measured in the Central Grand River.

The Grand River from headwaters to mouth

A study evaluating the carbon and nitrogen isotope delta in epilithon on the entire Grand River (Xiong 2014) revealed spatial differences in the isotopic composition of the river related to anthropogenic impact, namely flood control structures (km 70) and wastewater treatment plants (km 135 and 145, Figure 5.14).

Scarce data are available for the $\delta^{15}N$ and $\delta^{13}C$ of epilithon before the KTP upgrades. However some samples collected in 2009 showed a ^{15}N-depleted value (-1‰) below the urban area, whereas other locations (above and below the urban area) had $\delta^{15}N_{TN}$ between +4.7 and +7.3‰. The increase in $\delta^{15}N_{TN}$ observed after upgrades could be attributed to the upgrades of wastewater treatment plant in the Region of Waterloo (dashed lines in Figure 5.14). Increase in variability in $\delta^{15}N_{TN}$ after acidification of the sample has been reported for benthic invertebrates (Vafeiadou *et al*. 2013) and fish scales (Ventura and Jeppesen 2010); however,

not enough information is available for macrophytes and periphyton and it is assumed that acidification did not significantly affect the $\delta^{15}N_{TN}$ of the samples collected in this study.

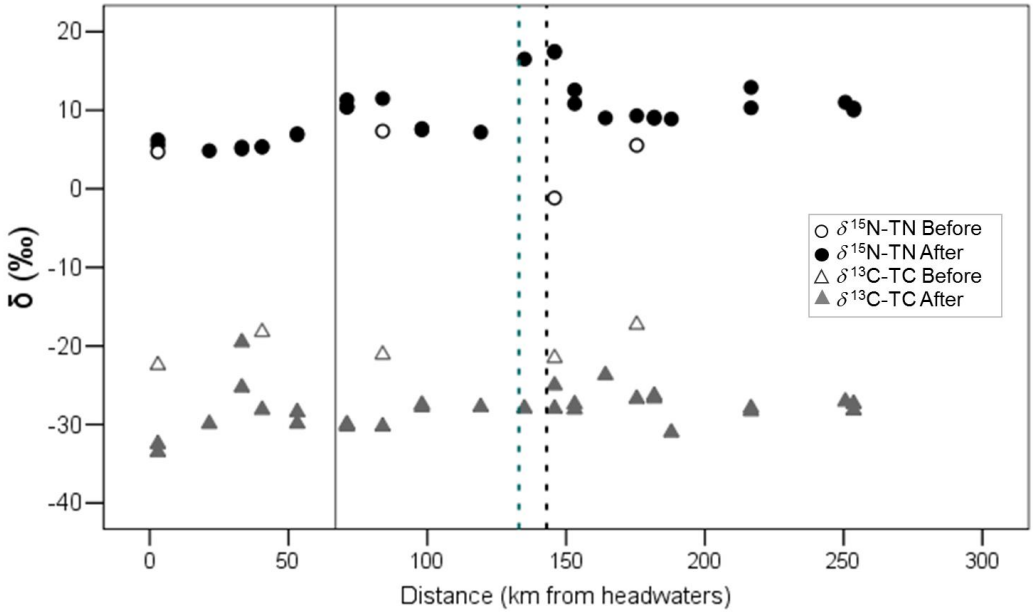


Figure 5.14 - Nitrogen ($\delta^{15}N_{TN}$) and carbon ($\delta^{13}C_{TC}$) isotope delta of epilithon along the Grand River. The solid line represents a flood control structure (Shand Dam); the dotted lines represent the two largest wastewater treatment plants in the Region of Waterloo (Waterloo and Kitchener, respectively). Data modified from Xiong 2014. *Before* upgrades data were collected in 2009, *after* upgrades data collected in 2013.

The differences found in $\delta^{13}C_{TC}$ values are mainly attributed to the differential treatment among samples: the samples collected and analysed before upgrades were not acid-washed, whereas samples collected in 2013 (after upgrades) were acid-washed, thus eliminating the carbonates from the sample. The smaller average C/N ratio of the acidified samples (7.8% ± 0.3) compared to the non-acidified samples (14.3% ± 3.2) supports the assumption that acidified samples represented the biomass without the carbonates.

CONCLUSION

With the data collected and analysed before and after the KTP upgrades, it is possible to relate the changes in the $\delta^{15}N$ of the macrophytes and periphyton to the $\delta^{15}N_{NH_4^+}$ and $\delta^{15}N_{NO_3^-}$ observed in the Central Grand River downstream of the KTP. Based in the isotopic separation

between the substrate ($\delta^{15}N_{NH_4^+}$) and the product ($\delta^{15}N_{macrophytes}$), it is proposed that discrimination against $^{15}NH_4^+$ was around -4.8‰ before upgrades and -12‰ after upgrades. However, due to the similar isotope delta between nitrate and some biomass, nitrate assimilation likely occurred. Thus; some macrophytes and periphyton in the Central Grand River downstream of the KTP can be used as a short-term environmental archive that incorporates the changes in the ammonium and nitrate isotopic composition of the effluent discharged into the Grand River. The $\delta^{15}N_{TN}$ of macrophytes and periphyton is proposed as a valuable tool that accumulates the changes in the water of a river receiving wastewater effluent in short periods that could be used complementary to water quality monitoring for assessing changes in the water chemistry of rivers and streams.

CHAPTER 6 - DISSOLVED INORGANIC NITROGEN DYNAMICS IN THE GRAND RIVER: A MULTI-YEAR APPROACH

INTRODUCTION

Essential elements in large demand that are required as basic constituents of all living organisms are referred to as macronutrients and include carbon, nitrogen, phosphorus, potassium, calcium, sulphur, silica and magnesium. Nitrogen and phosphorus are considered limiting nutrients for the generation of new biomass given that their availability influence the rates of primary production and the activity of heterotrophic organisms (Allan and Castillo 2007). An aquatic system is limited in nitrogen or phosphorus if the requirements are higher than the availability.

The global average nitrogen concentration in rivers oscillates from 0.12 mg N/L (total dissolved N) in ecosystems with little human influence, to close to 1 mg N/L in rivers with high human activity (Meybeck 1993). It has been estimated that 63% of the dissolved inorganic nitrogen (DIN) is originated by human activities. The DIN consists of three reactive nitrogen species: ammonium, nitrite and nitrate (Figure 6.1) and could be divided based on its origin into point-sources and non-point sources. The former includes wastewater treatment plants and septic systems with discreate discharges into rivers and streams; the latter reffers to agricultural and urban runoff and atmospheric deposition (Puckett 1995). Densely populated basins with high gross domestic product (GDP) and intensive agriculture usually have higher DIN yields (Dumont et al. 2005).

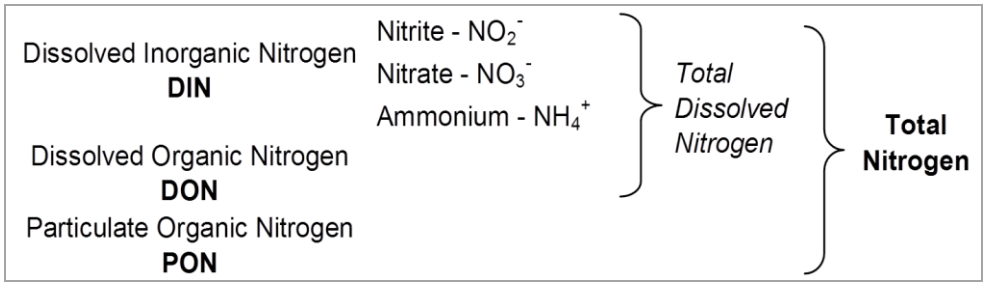


Figure 6.1 - Forms of nitrogen according to the molecular composition (organic and inorganic) and particle size (dissolved and particulate). Modified from Allan and Castillo (2007).

The magnitude of the impact of nitrogen non-point sources impacting a river or a stream is commonly associated to animal stocking density and excessive fertilization (Carpenter et al. 1998). However, there are other factors affecting the nutrient fluxes into aquatic ecosystems such as increased surface runoff (washing nutrients from the agro-ecosystems) and variable nutrient requirements by crops throughout the year (variable N assimilation). The weather variability among seasons and years modifies the inputs of nitrogen in rivers impacted by human activities such as the Grand River, given that high precipitation means higher river discharge; thus, higher dilution.

The capacity of a catchment to process, retain or export nitrogen depends on its in-stream productivity, nutrients availability (Allan and Castillo 2007), the channel morphology and the river discharge (Wollheim et al. 2001). In addition to dilution, the spatial variability of nitrogen in rivers and streams is the result of chemical and biological transformations such as nitrification, denitrification, immobilization, ammonia volatilization and assimilation, among the most important. Nitrogen can also be transiently stored elsewhere in the biosphere as biomass, adsorbed into sediments or modified by ground water/surface water interactions (Hinkle et al.2001). All external nitrogen inputs that are not removed or assimilated by the river are exported downstream to lakes and ultimatelly to the ocean.

In most aquatic ecosystems, the primary producers meet their nitrogen requirements by a combination of fixation, nitrogen return from sediments, atmospheric deposition and surface run-off (Schindler 2012). Consequently, additional inputs of nitrogen promote increasing primary productivity (such as algal blooms), nitrogen accumulation or nitrogen export. The central Grand River receives agricultural and urban inputs that represent an important increase in nitrogen and phosphorus (Loomer and Cooke 2011). An early characterization performed by MacCrimmon and Kelso (1970) on the Speed River and the south Grand River (Cambridge, Brantford and Caledonia) identified the wastewater treatment plants (WTP's) as sources of nutrients and potential threats to the trophic status of the river. WTP's were important sources of DIN and most of the sampling locations had high ammonium due to the lack of proper wastewater treatment. Since then, the Grand River has been thoroughly studied, included, but not limited to, nutrient uptake (Barlow-Busch et al., 2006), species composition along seasonal

and nutrient gradients (Metcalfe-Smith et al., 2000; Rott et al., 1998; Sreenivasa and Duthie, 1973), fish ecology (Cooke and Bunt, 1999), health risks from livestock rearing (Dorner et al., 2004), human activities (Lissemore et al., 2006) nitrogen (Rosamond et al., 2011) and phosphorus cycling (Hood 2012; Shaker 2014).

The Grand River watershed accommodates 30 municipal WTP's operating secondary and tertiary level treatments (Loomer and Cooke 2011). After treatment, the effluent is discharged back into the Grand River or its tributaries. The Region of Waterloo serves around half a million people, which represents approximately165 million litres per day (MLD) of wastewater discharged into the Grand River with more than 5500 kg N per day discharged into the Central Grand River (Region of Waterloo 2013).

The upgrades and expansion of facilities implemented on the Waterloo and Kitchener WTP's are expected to provide adequate wastewater treatment service capacity to an increasing population and maintain the aquatic ecosystem integrity. Before the upgrades, the two wastewater treatment plants had secondary treatment, grease and oil removed physically. Organic matter, phosphorus and ammonia was removed either microbially or chemically with a final chlorination step before discharging into the Grand River http://www.regionofwaterloo.ca/en/aboutTheEnvironment/Wastewater2.asp).

The Kitchener Wastewater Treatment Plant (KWTP) upgrades include a UV disinfection facility and effluent pumping station completed by early 2013, with a completely new secondary treatment plant completed by 2018, where full nitrification is expected (see Table 6.1 for the detailed timeline). The existing outfall and its diffuser would be removed and a new outfall (1950 mm concrete effluent pipe, 1800 mm diffuser) and tertiary filtration (phosphorus removal) has also been considered (Project File Report 2012, Region of Waterloo).

The Waterloo Wastewater Treatment Plant (WWTP) underwent a less extensive upgrading, with the objective of improving effluent quality to meet new criteria for discharge into the Grand River, improve energy efficiency, odour control, dewatering biosolids and increased treatment capacity (Wastewater treatment plant upgrade Newsletter 2, Aug. 2010, Region of

Waterloo). These upgrades are expected to avoid oxygen depletion during summer low flow conditions and decrease ammonium export.

Table 6.1 - Kitchener wastewater treatment plant upgrades timeline (Modified from Kitchener wastewater treatment plant upgrades Newsletter Issue 2, June 2013).

	2012	2013	2014	2015	2016	2017
Lagoon decommission and Digested sludge pumping						
UV disinfection						
Aeration systems (submerged air blower)						
Energy centre and Digestion						
Headworks, Plant 3 (Secondary treatment)						
Tertiary treatment and outfall						

Design, tender and award *Construction*

The multi-annual study completed in this thesis, provided a before-after approach to the DIN dynamics in a river receiving discharges from WTP's. The objective of this chapter is to identify and explain the intra-annual and inter-annual variations in the DIN observed in the Central Grand River in the context of the upgrades completed in the two largest WTP's of the Region of Waterloo. The working hypothesis is that the changes in operation of the WTP's impacted the DIN concentrations downstream of the central Grand River due to the large volume of effluent discharged from the WTP's into the Grand River; and such changes need to be related to the seasonal variability. It is important to stress that the data representing after upgrades is only one year; thus the interpretation provided here is limited.

MATERIALS AND METHODS

The Grand River watershed is commonly divided into northern till plains, central moraines and southern plains with lacustrine influence. The land use is mainly agricultural in the northern area, urban and industrial in the central and agricultural in the south area. The present chapter focused on a section of the Central Grand River, from West Montrose at the north (98 km form headwaters) to Brantford in the south (188 km from headwaters). This area sustains the densely populated urban cities of Guelph, Cambridge, Kitchener and Waterloo, together with several townships.

Sample collection and analysis

Two sampling strategies were used in order to capture a broad range of weather conditions, year-to-year variability and the effects of the WTP's upgrades:

a) Biweekly or three-week periodical sampling from 2008 and 2009 (collected by previous students and staff) and from 2010 to 2013 (collected during this research) at seven locations in the Central Grand River designated as semi-intensive sampling (see Table 6.2). The number of sampling events per year varied from 17 to 23.
b) One-day sampling campaigns for the whole extension of the Grand River, from headwaters to its mouth at Lake Erie, completed in 2007 and 2013 at different times of the year. In each event, 23 sites were sampled simultaneously by several teams (Figure 6.2). For comparison purposes at the watershed scale, 2007 represented *before* upgrades conditions, whereas 2013 represented *after* upgrades conditions, given that both events took place during late summer (September).

Data representing before upgrades were collected from 2010 to 2012, unless otherwise stated. After upgrades conditions are represented by data collected in 2013. All samples were collected assuming that the water column was well mixed. Sampling frequency was not always consistent among years; therefore, average values refer to time-weighted averages. Time-weighted averages were calculated considering the total of samples by season per year before and after WTP's upgrades (*see seasonal demarcation below*). River discharge (in m^3/s) was obtained from the Hydat Database (http://wateroffice.ec.gc.ca/) for the stations West Montrose (02GA034), Doon (02GA048) and Brantford (02GB001). The river discharge data (in m^3/d) for the location Bridgeport was obtained from the Grand River Information Network (GRIN, station 8665042). WTP's discharge and chemistry were obtained from the Region of Waterloo and from the Water and wastewater monitoring report (2007). Meteorological information was obtained from the archives of the University of Waterloo weather station.

Temperature, pH and electrical conductivity were measured *in situ* with multi-parameter probes (Hach multiprobe and YSI 560). Water samples for nutrients (ammonium, nitrate, nitrite, total dissolved nitrogen, dissolved organic carbon and phosphorus) were collected in HDPE bottles, filtered on-site (to 0.45 μm) and stored in ice (cooler). Winkler bottles were used for dissolved

oxygen measurement. Oxygen saturation was measured on the field with a multi-parametric probe or estimated with DOTABLES (water.usgs.gov/software/DOTABLES/). Nitrate and ammonium data was available since 2007, whereas nitrite data are available consistently since mid-2010. Additionally, total suspended solids samples (TSS) were also collected.

Table 6.2 - Sampling locations along the Grand River, southwestern Ontario, listed from north to south. Coordinates provided in decimal degrees. § indicate semi-intensive sampling locations.

Site Name	**Latitude**	**Longitude**	**Distance from headwaters** (km)
Dundalk	44.1458	-80.3422	2.9
Keldon	44.0377	-80.383	21.4
Leggatt	43.9674	-80.355	33.2
Above Grand Valley	43.923	-80.321	40.5
Below Grand Valley	43.8618	-80.2725	53.1
Shands	43.725	-80.344	71.0
Elora Gorge - Low Bridge	43.6766	-80.4459	83.9
§ West Montrose	43.5856	-80.4816	98.1
§ Bridgeport	43.4819	-80.4816	119.2
Waterloo WTP	43.4795	-80.4822	119.5
§ Victoria St.	43.4794	-80.4221	125.2
Freeport	43.4218	-80.4109	135.0
Kitchener WTP	43.401	-80.4322	140.3
§ Blair	43.3861	-80.3859	145.8
Parkhill Dam	43.3637	-80.317	153.1
§ Footbridge Rd.	43.3175	-80.3146	158.4
§ Glen Morris	43.2772	-80.3445	164.1
Paris	43.198	-80.382	175.5
Power Line Road	43.1741	-80.3531	181.8
§ Brant Conservation Area	43.1523	-80.3173	187.9
Newport	43.0994	-80.2404	216.6
Six Nations	43.0974	-80.0954	232.3
Sims Lock	43.0441	-79.909	250.6
York	43.0207	-79.8919	253.6
Cayuga	42.9496	-79.8606	263.1
Dunnville	42.9012	-79.619	288.1
Port Maitland	42.8599	-79.5756	295.7

Ammonium (NH_3+NH_4^+) and nitrite (NO_2^-) were measured by colorimetric methods (blue indophenol and sulphanilamide+azo dye, respectively), using a UV-VIS Beckman spectrophotometer and Smartchem 200 Autoanalyzer (±5% precision). Nitrate (NO_3^-) and other ions (chloride and sulphate) were analysed with an Ion Chromatograph (Dionex Corp. ±5% precision). Total Nitrogen was analysed by acid combustion in Apollo 9000 Combustion TOC/TN Analyzer (Teledyne Tekmar) and Shimadzu TOC-L Total Organic Carbon Analyzer with TNM-L Total Nitrogen Measuring Unit (precision ±0.3 mg C/N-DOC/TN L^{-1}). Dissolved inorganic nitrogen (DIN) was obtained by adding NO_2^-+NO_3^-+NH_4^+ for the period 2011-2013 (nitrite was not always analysed in 2010). Dissolved oxygen (D.O) was measured by Winkler titration (precision ±0.2 mg O_2 L^{-1}). For the purposes of this chapter, *load* refers to the mass that is added into the Grand River, *flux* refers to the mass that passes a sampling location and *export* represents the mass leaving the WTP's or the Grand River.

Concentrations and fluxes are reported and discussed were appropriate. Fluxes were calculated from the measured concentration at each location multiplied by the daily average discharge (m^3/d) obtained from the Hydat Database and the GRIN-GRCA; and reported as daily fluxes (m^3/day or mass of nitrogen/day). No annual fluxes or annual export are reported herein.

Statistical analyses and graphics were produced with JMP 5.1 and SPSS 13.0 (SAS Institute). Student's t test (α=0.05) were done for comparing the same location or parameter (DIN or DON) before and after upgrade conditions. Analysis of variance (ANOVA) was performed for TAN, NO_x (NO_2^-+NO_3^-) and TP in the KWTP effluent by seasons and to the DIN flux measured in the Central Grand River upstream and downstream of the KWTP effluent, before and after upgrades. ANOVA and the post-hoc test Tukey-Kramer HSD (q, α =0.05) were performed for TAN, NO_3^-, DON and temperature at selected location for comparing before and after upgrade conditions by season. Tukey-Kramer HSD was selected due to the fact that sample sizes were not equal.

Seasonal demarcation

Given that the astronomical definition of seasons does not reflect the observed conditions in the Grand River watershed, a hydrograph based on the Grand River annual average discharge is

considered to be a better descriptor of the weather variability, instead of precipitation and/or temperature. The terms “dry” and “wet” relative to the amount of precipitation are ambiguous when estimating the observed discharge, given that the Grand River has numerous flow control structures. Therefore, an hydrograph that normalize the observed flow (2010 to 2013) to low-flow and high flow conditions was used to create a customized seasonal demarcation for the analyses performed in this research.

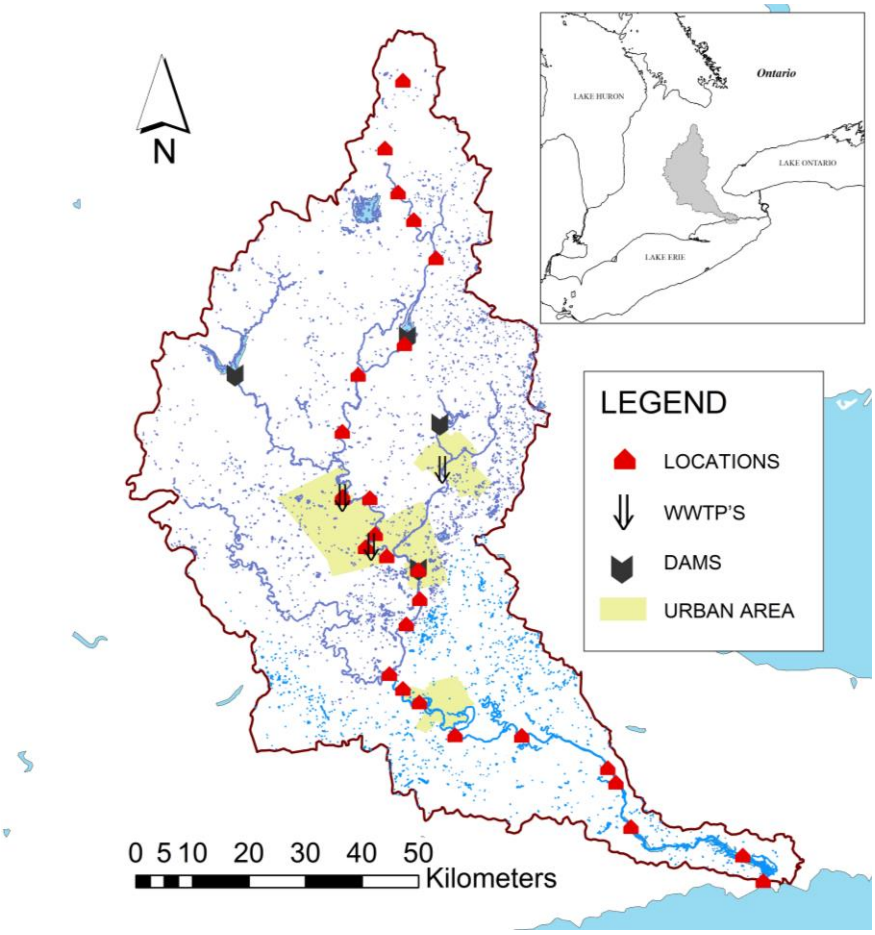


Figure 6.2 - Sampling locations along the Grand River, southwestern Ontario. The three largest wastewater treatment plants in the central Grand River and major dams are shown. Refer to Table 6.2 for details.

To normalize the hydrograph reported by the Water Survey of Canada, the observed discharge in each day of three years (in m^3/s, 2010 to 2012, n=3) was averaged and then divided by the average annual discharge observed in historical low flow years and historical high flow years.

Low flow and high flow annual average discharge by site were as follows: *Upstream (West Montrose)*=10.7/17.4 m^3s^{-1} (low/high); *Urban (Blair)*=19.2/32 m^3s^{-1} (low/high) and *Downstream (Brant Conservation Area)*= 44/81.6 m^3s^{-1} (low/high). Values that consistently grouped above, around or below 1.0 were considered to define the cut-off days (Figure 6.3 and Table 6.3). Although this seasonal demarcation seems arbitrary, there were additional criteria supporting the cut-off dates. For example, the normalized discharge during the spring melt was consistently above 1.0 for the 35 days assigned. For the spring-summer delimitation, the lowest air ambient temperature did not drop below 10° C. The winter demarcation corresponded almost completely with ice cover in the Grand River. The Table 6.4 provides meteorological information which assisted the interpretation of river conditions due to climate effects.

Table 6.3 - Seasonal demarcation based on normalized discharge for low flow and high flow conditions. Discharge (m^3/s) by day from 2010 to 2012 was averaged (Q_{obs}/Q_{avg}) and then normalized to low flow and high flow annual average discharge. *Upstream*=10.7/17.4 m^3s^{-1} (low/high); *Urban*=19.2/32 m^3s^{-1} (low/high) and *Downstream* 44/81.6 m^3s^{-1} (low/high). Data retrieved from Water Survey Canada.

Season	*Start date*	*End date*	*Cutoff (day)*	*Duration (days)*
Winter	*27-Dec*	*01-Mar*	60	*65*
Spring melt	*02-Mar*	*05-Apr*	*95*	*35*
Spring	*06-Apr*	*14-Jun*	*165*	*70*
Summer	*15-Jun*	*17-Oct*	*290*	*125*
Fall	*18-Oct*	*26-Dec*	*360*	*70*

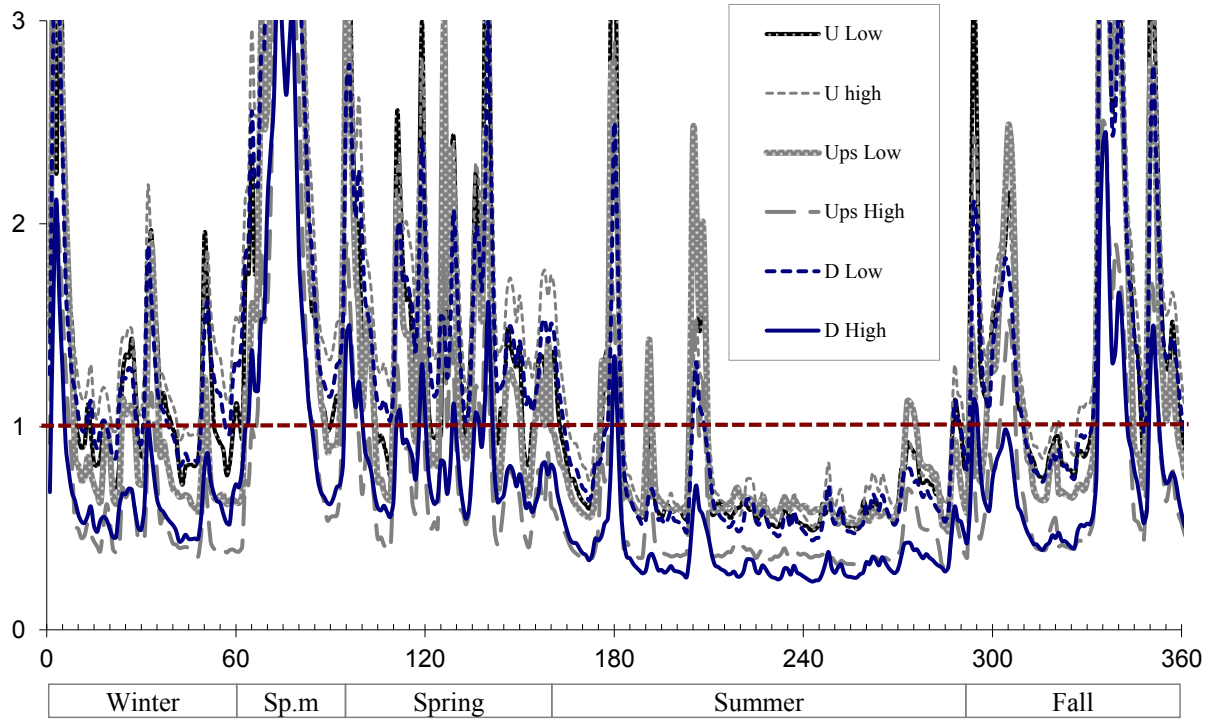


Figure 6.3 - Normalized discharge in the Central Grand River. Discharge (m^3/s) obtained from Water Survey Canada (2010-2012). Locations: *Ups*– West Montrose (98 km), *U* – Blair (146 km); *D* – Brant Conservation Area (188 km). Distance in kilometres from headwaters. *Sp.m* - spring melt. The Y axis has been cut for visualization purposes (peak value=8.6). Normalized discharge was obtained averaging daily discharge from 2010 to 2012 (Q_{obs}/Q_{avg}) and then normalized to low flow and high flow annual average discharge. Annual average discharge: *Upstream*=10.7/17.4 m^3s^{-1} (low/high); *Urban*=19.2/32 m^3s^{-1} (low/high) and *Downstream* 44/81.6 m^3s^{-1} (low/high).

Table 6.4 - Relevant meteorological information from 2007 to 2013. Data obtained from the University of Waterloo weather station (43.4738 N, 80.5576 W). Discharge (m^3/s) from Water Survey of Canada, stations West Montrose (WM-02GA034), Doon (BL-02GA048) and Brantford (BCA-02GB001). Long-term average discharge WM=16m^3/s (45 y), BL=31m^3/s (8 y) and BCA=59m^3/s (65 y).

Year	Comments	*Temp ºC*		*Historical avg ºC*	*Year avg ºC*	*Historical Precip. avg [mm]*	*Total year Precip. [mm]*	*Year snow avg [cm]*	*Total year snow [cm]*	*mean Q (m3/s) Water survey of Canada*		
		High	*Low*							**WM**	**BL**	**BCA**
2007	Dry year, total precipitation was three-quarters of the expected; six months recording below average precipitation. June was the driest month. Unusual late winter; the warmest October in about 60 years.	33.1	-28.6	*11.89*	12.46	*904*	671.9	*159.5*	104.5	9.7	18	41.1
2008	Very wet. 3rd highest annual total precipitation in the area since 1915. Seven months of the year with above average precipitation. Extreme precipitation in a day: 78 mm (July 11th)	30.4	-23.4	*11.89*	11.95	*904*	1159.5	*159.5*	332	22.5	46.3	95.5
2009	A cold year, 0.06ºC below average. Cold summer, Warm fall. First four months of the year were wet. Wet October and dry November	31.3	-28	*11.89*	11.67	*904*	899.1	*159.5*	128	17.1	32.5	66.2
2010	5th warmest year in history, 1.4ºC above average. Warmer spring. One week in July and one in August were notably above average. 11 days over 30ºC. Abundant rain: maximum one day rain: 65mm. 1 in 10 years precipitation. 46.1ºC highest humidex (July 7th)	33.1	-21.5	*11.89*	13.11	*904*	879.3	*159.5*	77.5	11.5	22	45.3

Table 6.4 (*cont*). Relevant meteorological information from 2007 to 2013. Data obtained from the University of Waterloo weather station (43.4738 N, 80.5576 W).Discharge (m^3/s) from Water Survey of Canada, stations West Montrose (WM-02GA034), Doon (BL-02GA048) and Brantford (BCA-02GB001). Long-term average discharge WM=16m3/s (45 y), BL=31m3/s (8 y) and BCA=59m3/s (65 y).

Year	Comments	*Temp ºC*		*Historical avg ºC*	*Year avg ºC*	*Historical Precip. avg [mm]*	*Total year Precip. [mm]*	*Year snow avg [cm]*	*Total year snow [cm]*	*mean Q (m3/s) Water survey of Canada*		
		High	*Low*							**WM**	**BL**	**BCA**
2011	Wet year, 4th wettest year since 1914. Wettest April ever. The first half of the year was 1°C colder than average; the second half was 2.5ºC warmer than average.	35.7	-28.8	*11.89*	12.6	*904*	1146.4	*159.5*	165.5	17.4	38	81.6
2012	Dry, consistently warm year. Hot March, over 7.5ºC warmer than average. The second warmest year since 1914. July 2011 to June 2012: the warmest 12 month period in the history. A large part of the year was drier than average. 56 mm one-day precipitation event (June 1st). 46.9ºC humidex (July 21st)	33.5	-18.3	*11.89*	14.31	*904*	782.7	*159.5*	86.5	10.7	19.2	44
2013	Year with more precipitation in 99 years record. 94.1 mm in one-day precipitation period (September). February, second snowiest record in the region. Late cold winter. 186 days without frost, longest frost-free season since 1915. 47.6ºC highest humidex (July 17th)	34.7	-24	*11.89*	11.92	*904*	1204.7	*159.5*	179	*n.a.*	*n.a.*	*n.a.*

RESULTS AND DISCUSSION

The KWTP upgrades and its role as nitrogen point source in the central Grand River

Due to the magnitude of the upgrades implemented at the KWTP and the sampling strategy implemented in this research, large part of the results discussed here refer to before and after upgrades at the KWTP; however, it is important to mention that the urban effects represent the DIN from the two largest WTP's (Waterloo and Kitchener) in addition to other WTP's located north of the cities of Waterloo and Kitchener in the central section of the watershed.

The KWTP currently handles 65,000–70,000 m^3/d; however, it could operate above 122,000 m^3/d for short periods of time in case of extreme events. On average, 70000 m^3 per day of treated sewage are disposed into the central Grand River (Region of Waterloo 2014). The upgrades of the KWTP were completed at different times; however, the critical date of changes in the operation was set on August 1st 2012, when aeration tanks of the new secondary treatment train began to operate. Between August 1st and August 23rd 2012, the ammonium concentration in the KWTP effluent dropped from ~25 to ~5mg N-NH_4^+/L (Region of Waterloo 2014). During the second half of 2012, operational irregularities at the KWTP were still observed, which resulted in final effluent with ammonia concentrations between 3 and 20 mgN-NH_4^+/L in late summer and in the fall of 2012. Finally, as of January 4th 2013, the new train for secondary treatment started full operation releasing an almost fully nitrified effluent. Ammonium concentration below 5 mg N-NH_4^+/L has been observed in the effluent since May 2013.

The nitrogen load from the KWTP was different among years, lower loads were observed before upgrades (1681±216 kg N-DIN per day) than after upgrades (1905±236 kg N-DIN per day, Student's t=2.66, α=0.01. Figure 6.4). The nitrogen discharged from the KWTP before upgrades increased approximately 220 kg N per day after completion of the upgrades. This change in nitrogen load might be attributed to higher loss of $NH_{3(g)}$ before the upgrades, the increase in volume treated and the recirculation of the centrate, the low-volume high-concentration liquid result of biosolids dewatering.

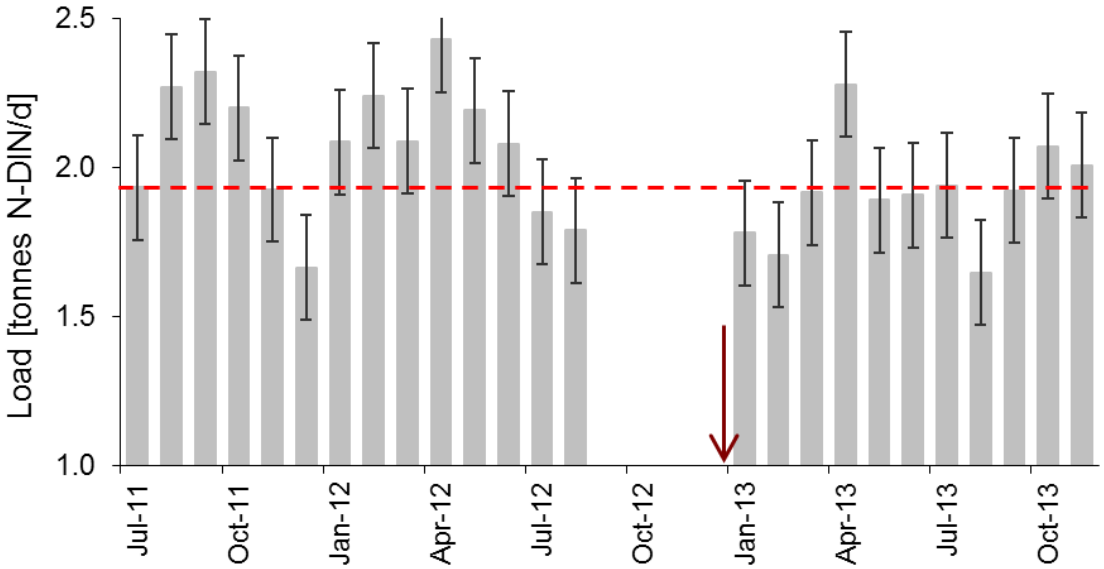


Figure 6.4 - Dissolved inorganic nitrogen (DIN) load in tonnes per day released to the Grand River from the Kitchener wastewater treatment plant before (2011-2012) and after upgrades (2013). Monthly average of daily values ± 1 s.d. The arrow indicates the date when all secondary treatment tanks were commissioned. Dashed line represents the median of DIN load from July 2011 to November 2013. Data provided by Region of Waterloo and obtained from the Water and wastewater monitoring report (2012).

The quality of the effluent after upgrades was variable throughout the year 2013. Several water quality parameters of the effluent were significantly among seasons. The NH_4^+ in the effluent was higher in winter than the rest of the year ($F=22.28$, $p<0.0001$, $df=41$), assumed to be a result of depressed biological activity due to the low water temperature, in additon to less ammonia volatilization. $NO_2^-+NO_3^-$ was higher in the spring, summer and fall compared to winter $NO_2^-+NO_3^-$ ($F=10.027$, $p<0.0001$, $df=38$), supposed a result of more intense nitrification. TP was higher in spring melt than in the summer or fall ($F=2.67$, $p=0.045$, $df=47$), presumably due to greater surface runoff.

The relative contribution of the effluent from the two largest WTP's over the Central Grand River flow is shown in Table 6.5. In years with flow below historical normal (*i.e* dry years), the WWTP effluent comprised 4% of the summer river discharge. The effluent discharge from the KWTP represented around 8% of the river discharge. On the other hand, during years with flow above historical normal (wet years), the effluent of the KWTP was between 3 and 5% of the Grand River discharge. That is to say that the effluent discharged into the Grand River during

wet years was more diluted and the DIN concentration measured in the Grand River were lower than dry years, when dilution is reduced.

Table 6.5 - Wastewater treatment plant discharge to Grand River discharge average ratio (*WTP/River*, in percentage for both WWTP and KWTP) in years with below- and above historical normal flow in the Central Grand River. WTP's discharge provided by the Region of Waterloo; Grand River discharge obtained from Water Survey Canada (Doon-02GA048) and GRCA (Bridgeport GRCA-8665042). WWTP-Waterloo wastewater treatment plant, KWTP-Kitchener wastewater treatment plant.

	WWTP		***KWTP***				
	Flow below historical normal			**2012 - flow below historical normal**		**2013 - flow above historical normal**	
Month	***n***	Avg WTP/River ratio ± s.d. (%)	Month	*n*	Avg WTP/River ratio ± s.d.(%)	*n*	Avg WTP/River ratio ± s.d.(%)
January *	4	1 ± 0.4	January	*5*	3 ± 2	*31*	3 ± 2
February *	5	2 ± 0.7	February	*4*	3 ± 1	*28*	3 ± 1
March *	5	4 ± 2	March	*5*	3 ± 2	*31*	2 ± 1
April *	4	2 ± 1	April	*4*	7 ± 1	*30*	2 ± 1
May *	5	4 ± 0.6	May	*5*	8 ± 1	*31*	5 ± 2
June *	4	4 ± 2	June	*4*	8 ± 1	*30*	3 ± 2
July §	4	4 ± 1	July	*31*	8 ± 1	*31*	3 ± 2
August §	5	3± 0.8	August	*30*	8 ± 1	*31*	5 ± 2
September §	4	4 ± 0.9	September		*n.a.*	*30*	4 ± 2
October §	4	2 ± 0.8	October		*n.a.*	*31*	2 ± 1
November §	4	2 ± 0.5	November		*n.a.*	*30*	2 ± 1
December §	5	1 ± 0.7	December		*n.a.*		*n.a.*

§ 2011, * 2012

Given the projected increase in population to around one million people living in the Grand River watershed by 2030 (Region of Waterloo 2012), the Grand River ecosystem integrity could be at risk due to increases in sewage inputs. For example, during 2011 and 2012, the effluent from six of the WTP's discharging into the central Grand River and the tributary Speed River at Guelph, comprised around 5.5 tonnes of nitrogen per day (Table 6.6). As the population increases, nitrogen loads from the WTP's will also increase, which will cause high DIN concentration at critical periods in critical locations of the central and south Grand River.

During the summer of the four years monitored, the N discharged into the Central Grand River by the two largest WTP's in the Region of Waterloo (Waterloo and Kitchener) represented an

increase between 35% and 76% of the N passing between the location Bridgeport (upstream of the urban area, 119 km form headwaters) and the location Blair (5700 m downstream of the KWTP, 145.8 km from headwaters; Table 6.7). These N inputs were proportionally more important during the dry years (2011 and 2012) that the wet years (2010 and 2013). The calculated N fluxes at the two locations compared represent the net N addition of the two largest WTP's into the Central Grand River between the location upstream of the urban area and the location represents most of the urban impacts (*i.e.* accounts for the N assimilation).

Table 6.6 - Dissolved inorganic nitrogen loads from selected wastewater treatment plants discharging into the Central Grand River. [1] Data provided by the Region of Waterloo; 2011 to 2013. [2] Data from Guelph wastewater treatment plant annual report 2011 and 2012,http://guelph.ca/living/environment /water/ wastewater/. [3] Effluent Chemistry 2011-2012; discharge estimated from the rated flow based the 85th percentile per capita flow over the years 2000-2005 (Region of Waterloo Water & Wastewater Monitoring Report 2007).

Wastewater Treatment Plant	**load Kg N day $^{-1}$ year average (±S.E)**	**Min - Max Kg N day^{-1}**
[1] Waterloo	1083.8 ± 59	707 - 2158.7
[2] Guelph	1400.1 ± 116.5	1125.2 - 1603.3
[3] Hespeler	144.7 ± 11.6	101.8 - 204
[3] Preston	324.5 ± 5	246.8 - 369.5
[3] Galt	866.3 ± 12.2	726.8 - 1030.9
[1] Kitchener *(before)*	1674.1 ± 216.3	1052.5 -1986.9
[1] Kitchener *(after)*	1909.7 ± 116.9	767.1 - 3944.9

The effects of the KWTP upgrades were also observed on the dissolved oxygen concentrations. The chemical oxygen demand (COD) of the effluent after upgrades was reduced to less than 5% of the COD before-upgrades (Region of Waterloo effluent discharge quality data, unpublished), which in turn improved the dissolved oxygen concentration downstream of the effluent as no oxygen was required to oxidize ammonium.

Table 6.7 Dissolved inorganic nitrogen fluxes (kg N per day) in the summer of 2010 to 2013 measured upstream of the urban area of the Region of Waterloo (Bridgeport, 119 km form headwaters) and the location 5700 m downstream of the urban area of the Region of Waterloo (Blair, 145.8 km form headwaters). WTP's discharge and water chemistry provided by the Region of Waterloo. Water chemistry at the location 5700 m downstream of the KWTP measured as described in Materials and methods. River discharge at the location 5700 m downstream of the KWTP obtained from the Water Survey of Canada (02GA048)

Year	Location	Summer flux (kgN/d)	% increase N from WTP's
2010	Upstream	2122	50%
	5700 m downstream KWTP	4254	
2011	Upstream	1383	44%
	5700 m downstream KWTP	2471	
2012	Upstream	583	76%
	5700 m downstream KWTP	2444	
2013	Upstream	2483	35%
	5700 m downstream KWTP	3812	

Finally, upgrades of the WTP's in the Region of Waterloo would also assist in reducing some emergent contaminants of concern. For instance, municipal secondary treatment plants with UV disinfection protocols can remove antibiotics (Andersen *et al*. 2003). Estrogen has not been detected in the Grand River as a result of UV disinfection (Mark Servos, *pers*. *comm*.). Additional measurements for recognizing and identifying wastewater discharges impacts include coliform bacteria count (Brosnan and O'Shea 1996) and bacterial biodiversity (Sonthiphand *et al*. 2013).

Seasonal DIN changes in the Central Grand River before and after the WTP's upgrades

The general trend for all seven sampling locations in the Central Grand River before and after upgrades of the WTP's showed a consistent seasonal trend in DIN concentrations, being elevated during high flow periods (*i*.*e*. spring melt and fall) and low temperature seasons (*i*.*e*. fall and winter). West Montrose and Bridgeport, upstream of the urban area (and representing inputs from agriculture activities) had high DIN concentrations (≥5 mg N/L) in the late fall and winter period, particularly rising above 5 mg N/L during spring melt in wet years (2008 and 2013). Samples between 2 and 3 mg N/L were commonly observed during spring and summer.

The sampling locations Victoria and Blair showed similar trends; however, Blair had extended periods with DIN concentrations above 4 mg N/L, due to its proximity to the KWTP (5700 metres downstream of the effluent). Concentrations above 5 mg N/L were observed in late fall in 2012, over winter and spring 2013. This increase in N is likely a result of increased nitrate release from the KWTP after upgrades. DIN above 5 mgN/L during winter was also observed at Brantford, 40 km downstream of the KWTP. Nitrite (NO_2^-) was observed to be particularly high in the sampling location Blair, five kilometres below the KWTP (annual average 0.2 ±0.05 mgN-NO_2^-/L before upgrades). Contour plot for all semi- intensive sites are showed in Appendix I.

The locations north of the urban area (West Montrose and Bridgeport) showed consistently low ammonium throughout the year, with some punctual increases (≤0.6 mgN-NH_4^+/L) in late summer 2012. High ammonium is assumed to be the result of manure application in agricultural fields.

The location Blair (downstream the urban area, 146 km from headwaters) represents the sampling point where the impacts of the Waterloo and Kitchener WTP's are observed. This location had around 0.4 mgN-NH_4^+/L (average concentration before upgrades, 2010 to 2012), with peak values of 2 mgN-NH_4^+/L in spring 2010 and late summer 2012. Ammonium was particularly high in this sampling location on the summer nights due to the low dissolved oxygen and null photosynthetic oxygen evolution. During the year 2012, several samples collected at Blair during the night had ammonium concentrations above 1 mgN-NH_4^+/L.

The south end of the Central Grand River (Brantford – 204 km from headwaters) collects the effects of both agricultural and urban land uses and also the entrance of the tributary Speed River. Ammonium above 0.5 mgN-NH_4^+/L was seldom observed throughout the year, which suggests that large part of the ammonium was assimilated in the river.

Nitrate was commonly high during winter at West Montrose and Bridgeport (2.5 to 3.5 mg N-NO_3^-/L), and was slightly higher in wet years (i.e., precipitation above average and high discharge). High NO_3^-in the central Grand River is attributed to agriculture and inputs from

other WTP's upstream the Region of Waterloo (Figure 6.5). These locations showed important inter-annual variations in NO_3^-concentrations: wet years (2008, 2009, 2011 and 2013) had higher NO_3^-concentration, considered the result of local increases in surface runoff. During a warm year (2010), an almost uniform increase of 0.5 mgN-NO_3^-/L in all samples collected was observed at Bridgeport, attributed to the contribution of agricultural tributaries *(e.g.* Conestogo River) that drain intensive agricultural sub-catchments (Figure 6.5, BR 119 km). Summarizing, upstream of the KWTP there are important inter-annual changes in addition to the pronounced seasonal cycles that resulted in differences between years even when accounting for seasonal cycles.

Downstream of the KWTP, the highest NO_3^-concentration observed at Blair was 7.1 mgN-NO_3^-/L in winter 2008 (see Figure 6.5, BL 146 km). The reach of the Grand River downstream of the KWTP has been identified of particular concern due to low dissolved oxygen concentration during the summer (below 4 mg DO/L in the summer) failing to meet the Provincial Water Quality Objectives (Wastewater Treatment Master Plan Report - Region of Waterloo, 2007).

Further downstream, at Brantford, the NO_3^- annual average (4.09 mgN-NO_3^-/L) and annual median (3.88 mgN-NO_3^-/L) after upgrades were not different than before upgrades (Tukey-Kramer HSD). Cold and dry years maintained relatively high concentrations; low temperatures imply depressed biotic activity and dry years represent reduced dilution. It was expected that groundwater discharges will promote recovery of the water quality (MacCrimmon and Kelso 1970). However, some groundwater samples collected upstream of Brantford before upgrades had nitrate concentrations between 0.05 to 5.0 mgN-NO_3^-/L (median=3.8 mgN-NO_3^-/L, Westberg 2012); thus, the dilution expected by groundwater might not achieve its objective.

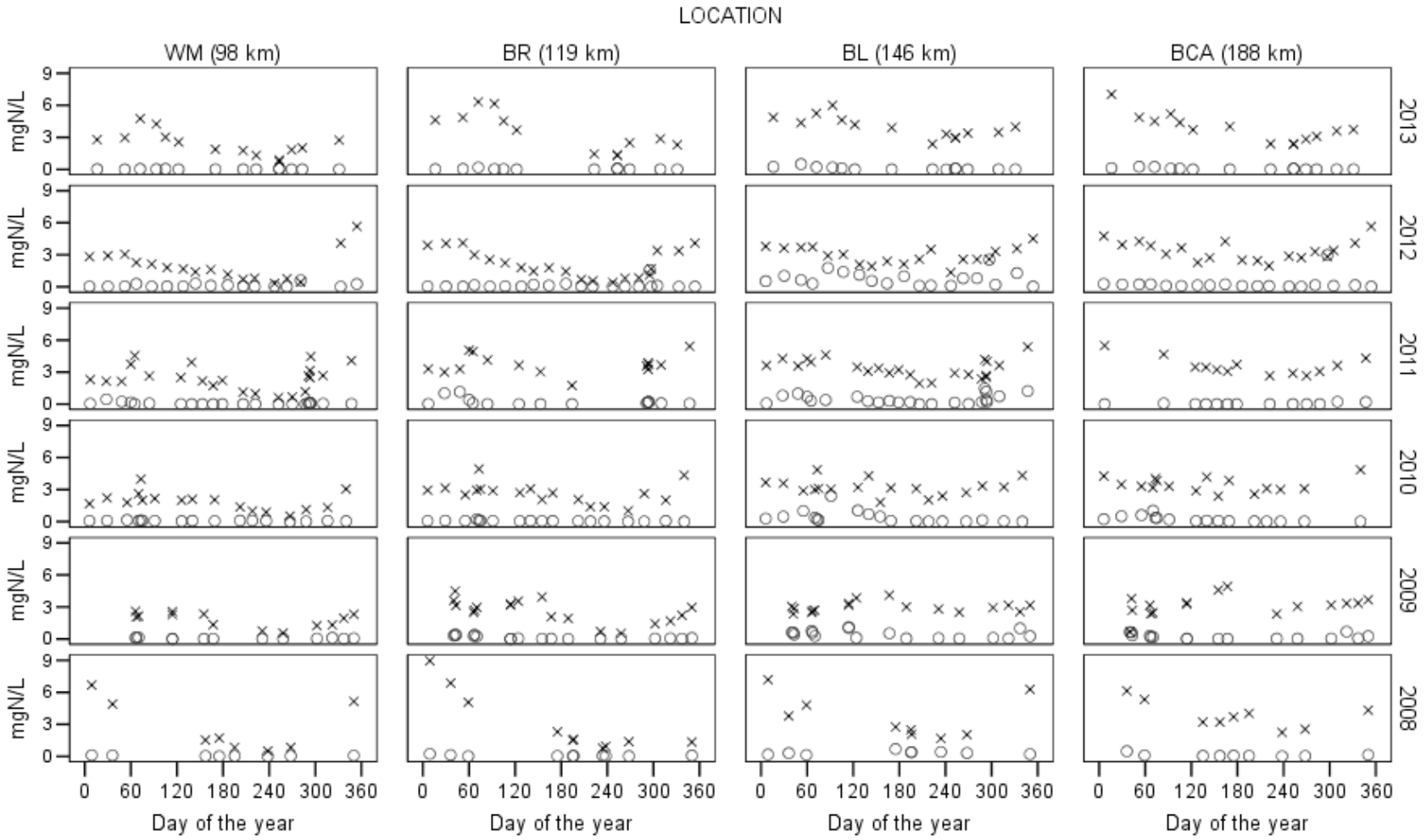


Figure 6.5 - Ammonium (o) and nitrate (×) concentrations (mg N /L) before (2008-2012) and after (2013) Kitchener wastewater treatment plant upgrades. Selected sites represent upstream (WM and BR) and downstream (BL and BCA) locations relative to the two largest wastewater treatment plants in the Region of Waterloo.

The NO_3^- measured in the Central Grand River downstream of the Region of Waterloo (Brantford) represents not only WTP's discharges, but also agricultural impacts and surface runoff from upstream tributaries. According to the Grand River Watershed Water Management Plan (2014), four sources of nitrate upstream of the urban area of Central Grand River have been identified: agricultural creeks (contributing with almost 50% of the total nitrate loads), the Conestogo River (40%), the Shand dam (8%) and septic systems (2%). Thus, the agricultural NO_3^- has to be considered when estimating the net effects of the WTP's upgrades.

Increases in nitrate concentrations in the Central Grand River downstream of the urban area are expected to occur as a result of WTP's upgrades. The magnitude of nitrate increase based on the Grand River Simulation Model (GRSM, a one-dimensional, dynamic nutrient and dissolved oxygen water quality model) was predicted to be around 1.1 mgN-NO_3^-/L relative to upstream locations. The modeled scenarios for summer low flow consider simultaneous increases in cumulative upstream sources (i.e. increase in population served by WTP's) and a 10 to 25% reduction in non-point sources (GRCA Water Management Plan Assimilative Capacity Working Group 2012). Thus, predicted nitrate concentration in 2031 during low flow conditions have been assumed to be around 4 mgN-NO_3^-/L right downstream the KWTP effluent and downstream of the Speed River discharge (75 percentile concentration).

Nitrate concentration is expected to decrease to around 3 mgN-NO_3^-/L at Brantford (47 km downstream of the KTP effluent) due to dilution, biological uptake and denitrification GRCA Water Management Plan Assimilative Capacity Working Group 2012). However, in the fall of 2013, nitrate in the Grand River downstream of the KWTP was between 3.3 and 4 mg N-NO_3^-/L, surpassing the target value of 3 mg N-NO_3^-/L. The data collected in this research also showed reductions in NO_3^- downstream of the KWTP (see Figure 6.5); however, not always below the NO_3^- target value. Thus, with the samples collected between 2010 and 2013, the nitrate increase used for modelling purposes (1.1 mgN-NO_3^-/L) is likely to be surpassed in the summer of dry years.

Statistical analysis performed on the time-weighted average concentration by seasons showed that the upgrades in the KTP represented lower-than-before ammonium in most seasons (all but

winter, mgN-NH_4^+/L at BL, Table 6.8) downstream of the KTP effluent and higher-than-before nitrate at Blair and Brantford Conservation Area (mgN-NO_3^-/L at BL and BCA, Table 6.8). However, some of the changes in nitrate and ammonium concentrations might not be directly related to upgrades of the WTP's. For example, there were significant differences in ammonium and nitrate concentrations upstream from the WTP's, which suggested that some changes in the water chemistry of the Grand River could have not been completely related to the upgrades on the WTP's in the Region of Waterloo. The year 2012 was not considered during the ANOVA, because that year had large oscillations in the effluent quality due to the sequential decommissioning-commissioning of the aerator tanks.

Table 6.8 - Ammonium and nitrate time-weighted average concentrations by season before (2010-2011, n=392) and after (2013, n=87) Kitchener wastewater treatment plant upgrades at three locations in the Central Grand River. Seasonal demarcation based on normalized discharge. Horizontally different letters represent statistical differences before and after upgrades. ANOVA ($F=11.64$, $p<0.0001$), post-hoc test Tukey-Kramer HSD ($p<0.05$). BR is located upstream of the WTP's effluent and BL and BCA are located downstream the WTP's effluent.

		mg N-NH_4^+/L		mg N-NO_3^-/L	
Location	***Season***	***Before***	***After***	***Before***	***After***
	Winter	0.09 *(a)*	0.06 *(b)*	3.32 *(a)*	4.74 *(b)*
	Spring melt	0.09 *(a)*	0.10 *(a)*	3.80 *(a)*	6.24 *(b)*
BR [119 km]	Spring	0.03 *(a)*	0.01 *(a)*	3.19 *(a)*	4.05 *(a)*
	Summer	0.01 *(a)*	0.01 *(a)*	1.82 *(a)*	2.44 *(a)*
	Fall	0.20 (*a*)	0.10 *(a)*	3.73 *(a)*	2.57 *(a)*
	Winter	0.48 *(a)*	0.32 *(a)*	3.71 *(a)*	4.61 *(b)*
	Spring melt	0.63 *(a)*	0.19 *(b)*	3.76 *(a)*	5.63 *(b)*
BL [146 km]	Spring	0.64 *(a)*	0.04 *(b)*	3.19 *(a)*	4.41 *(b)*
	Summer	0.12 *(a)*	0.02 *(b)*	2.71 *(a)*	3.10 *(a)*
	Fall	0.86 *(a)*	0.27 *(b)*	3.83 *(a)*	3.68 *(a)*
	Winter	0.23 *(a)*	0.13 *(a)*	4.17 *(a)*	5.45 *(b)*
	Spring melt	0.39 *(a)*	0.14 *(a)*	3.69 *(a)*	4.85 *(b)*
BCA [204 km]	Spring	0.04 *(a)*	0.03 *(a)*	3.27 (*a*)	4.07 *(b)*
	Summer	0.01 *(a)*	0.01 *(a)*	3.04 *(a)*	3.05 *(a)*
	Fall	0.21 *(a)*	0.02 *(a)*	3.96 *(a)*	3.67 *(a)*

As a result of the WTP's upgrades, there was a decrease in 75% of the ammonium and 50% of the nitrite concentrations measured in the Central Grand River below the urban area (Table 6.9). The decrease in half of the observed nitrite is the result of less ammonium oxidation in the

water column, which also improved the dissolved oxygen in the river, being 33% higher after upgrades. On the other hand, there was an increase in 23% of the nitrate measured in the river, due to the increase in nitrate discharge from the KWTP. Comparing only the before and after upgrades concentrations, there was an observed decrease in 19% of the TN measured in the Central Grand River 5700 m downstream of the KWTP effluent (Table 6.8). However, it is important to mention that some of these changes in concentration could be the result of increase dilution in 2013 (a wet year) compared to the pre-upgrades years (2010 to 2012).

Table 6.9 - Changes in time-weighted annual average concentration of water quality parameters at the location Blair, 5.7 km downstream of the Kitchener wastewater treatment plant. Values with different letters are significantly different (post-hoc test Tukey-Kramer HSD, $p<0.05$). Pre-upgrades (2010-2011, n=46), during upgrades (2012, n=20) and post-upgrades (2013, n=17). Samples represent daytime concentrations.

	Pre-upgrades (2010-2011)	*During upgrades (2012)*	*Post-upgrades (2013)*	*% Change*
NH_4^+ (mg N/L)	0.44 (a)	0.79 (b)	0.11 (c)	-75%
NO_2^- (mg N/L)	0.28	0.19	0.14	-50%
NO_3^- (mg N/L)	3.25 (a)	3.0 (b)	4.01 (c)	+23%
DIN (mg N/L)	3.96	3.89	4.17	+5%
TN (mg N/L)	5.31 (a)	4.72 (ab)	4.29 (b)	-19%
TP (μg P/L)	93.69	67.19	62.14	-33%
SRP (μg P/L)	33.4 (a)	80.4 (b)	41.15 (ab)	+23%
Cl⁻ (mg Cl/L)	83.81 (a)	91.77 (b)	46.93 (c)	-44%
D.O (mg O_2/L)	8.27 (a)	8.45 (a)	11.03 (b)	+33%

Similar improvements in water quality after wastewater treatment plant upgrades were achieved in temperate rivers. For example, upgrades in WTP's discharging into the New River estuary (North Carolina) accomplished 81% reduction in ammonium, 28% reduction in nitrate and 49% reduction in orthophosphate, with the consequent increased in dissolved oxygen (Mallin *et al*. 2005). The Table 6.8 shows a comparison of changes in nutrients concentration before, during and after upgrades at Blair, the location closest to the KWTP's effluent, which represents the most important impacts of the KWTP in the Central Grand River and is location part of the Provincial Water Quality Monitoring Network (PWQMN). The DIN concentration did not

change greatly; however, long-term exposure to high nitrate concentration is likely to represent an important impact on sensitive aquatic organism (Canadian Council of Ministers of the Environment, 2010) in addition to the 10 mgN-NO_3^-/L limit for drinking water (Health Canada 2013).

The changes in the water chemistry of the Grand River at Blair (downstream of the KTP effluent) could have an effect on the nutrient ratios of the dissolved species (DIN:SRP). The N:P ratio before was 110 and 80 after the KTP upgrades. The changes in DIN, P (and Cl^-) observed in the Central Grand River can be related to dilution rather than actual changes to the WTP's upgrades; nonetheless, it is important to closely monitor the nutrient ratios to understand and potentially predict changes in the trophic status of the Grand River.

Intra-annual and inter-annual variations

Intra-annual and inter-annual variations in DIN concentration in the Central Grand River could be attributed to changes in the water temperature, the agricultural growing cycle and variable flow among seasons and years.

The water temperature in the Central Grand River was different among years (ANOVA F=2.68, p=0.05, df=103). The water was cooler in the summer of 2013 (avg=17.7 ºC) than the summer of 2010, 2011 or 2012 (Tukey-Kramer HSD q=2.61, p=0.042). The difference in 3 ºC between years likely is not as important as the seasonal differences in water temperature. The water temperature of the Central Grand River varies drastically among seasons, oscillating between 0 °C in winter to as high as 26 °C in summer. Low temperature (rather than high temperature) affects the biotic removal mechanisms. For example, nitrification's optimum temperature is 15°C, decreasing as the temperature is lower (Charley *et al*. 1980). The fact that nitrate inputs from agricultural catchments during the non-growing season occur simultaneously with low temperature in the Grand River is likely to facilitate high nitrate (and ammonium) concentrations in the Grand River. The seasonal effect of water temperature is observed as nitrate and ammonum concentrations greater than 5 mg N-NO_3^-/L and greater than 0.5 mg N-NH_4^+/L from fall until mid-spring (over winter), concurrent with water temperatures lower than 15ºC (Figure 6.6). Temperature and variable flow can combine under special circumstance to

enhance or reduce primary productivity. For example, some diatoms show higher nitrate uptake at low temperatures (Lomas and Glibert 1999). However if the TSS is high due to fast flow, light penetration would be low and primary productivity will be reduced.

Dentritification is also influenced by temperature. Rosamond *et al*. (2011) found intense denitrification (and maximum N_2O concentration) during summer with high water temperature and low dissolved oxygen conditions. Pfenning and McMahon (1997) observed that lowering the temperature to 4°C resulted in an approximately 77% decrease in the N_2O production rates. 10ºC is the optimal uptake temperature for the majority of the mesophilic biota, reductions in temperature resulted in reduced assimilation of nitrate in several algal and bacterial of different physiological types (Reay *et al*. 1999). The effect of temperature on the metabolism has been attributed to alterations in the physical properties of cell membranes, an overall decrease of fluidity, inactivation of proteins and decreased enzyme affinity (Clarkson *et al*. 1988; Reay *et al*. 1999).

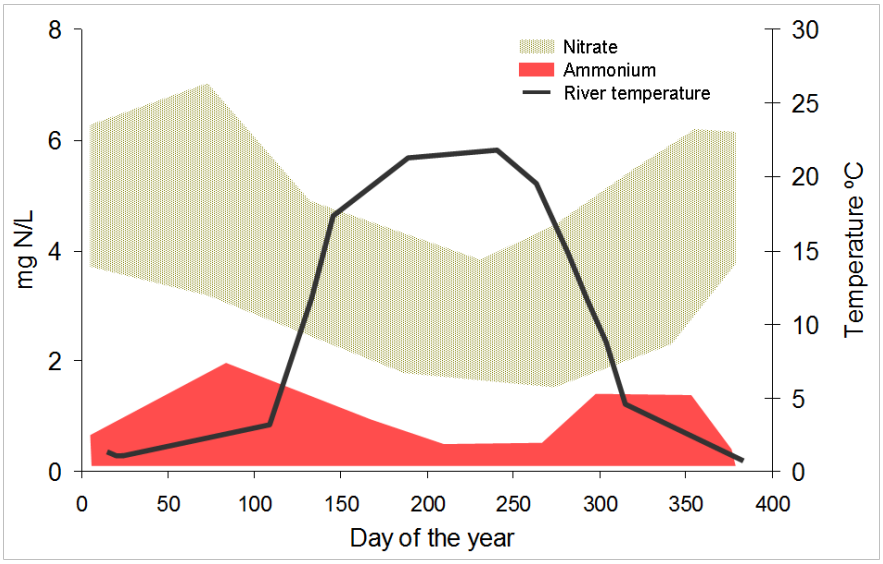


Figure 6.6 - River temperature and nitrate and ammonium trends at seven locations in the Central Grand River, covering from 98 to 204 km from headwaters (*semi-intensive sampling locations*). Nitrate (green) and ammonium (red) polygons include data collected from 2008 to 2013 (n=627), temperature trend includes data from 2009 to 2013 (n=578).

The second intra-annual effect in variable DIN concentration in the Central Grand River is related to the active growing season of crops. During summer, ammonium and nitrate

concentrations usually decrease when biological assimilation by crops is more intense (during the growing season), thus not contributing significantly to the nitrate measured in the Central Grand River. When the crops are harvested and there is not active nutrients uptake in the agricultural fields, the DIN in streams and the river increase as a result of active tile drainage and runoff. The increase in DIN concentration in running waters is particularly noticeable during the high water table season (fall, winter and spring); thus large loads of nitrate being mobilized from the agricultural sub-catchments into the tributaries of the Grand River (Cummings 2014).

The third intra-annual effect is the variable flow among seasons (see *Seasonal demarcation*). Over winter, some sections of the Grand River are covered with ice that melt during March and April (the spring melt) comprising a very important increase in discharge (up to 10-fold increase in discharge). On the other hand, summer is the season with the yearly minimum discharge (*i.e*. annual base flow). This variability in river discharge promotes variable dilution of all the nutrient inputs into the Grand River such as release from reservoirs, surface runoff and wastewater effluent among the most important.

Variability in the river discharge is even more important at the *inter-annual* scale, given that it clearly differentiate between years with annual average discharge *above* historical normal (wet years) and years with annual average discharge *below* historical normal (dry years). The river discharge is probably the most important parameter affecting the water quality of a river receiving wastewater effluent, given that it enhances or limits dilution, regulates the sediment movement, enhances or limits primary productivity and influencing the biotic community structure, among the most important (Junk *et al*. 1989). The inter-annual differences in DIN concentrations (and other elements such as phosphorus and chloride) in the Grand River are assumed to be due to the variable *effluent:river* ratio, which results in different dilution rates of the WTP's effluent relative to the river discharge. Above-average annual river flow is the measurable expression of above average annual precipitation, driving high groundwater discharge and increased water release from dams. Water release from dams is one of the most important factors regulating base flow in the Grand River, given that seven flood regulation

structures in the watershed regulate river flows. The thickness of the snowpack also contributed to high soil saturation and increased rier flow due to surface runoff.

As proposed in seasonal demarcation, wet or dry years are defined by river discharge (m^3/s). Wet years such as 2008, 2011 and 2013 had high river discharge which represent larger fluxes of nutrients, but also increased dilution and increased flow velocity that could influence the metabolic rates and the thickness of the diffusive layer in microbial communities and plant surface (Biggs 1996). Dry years such as 2007 and 2012 had reduced dilution, thus leading to higher concentrations observed in the river, in addition to the variable quality of the effluent discharged from the WTP's.

Flushing and dilution of nutrients due to changes in discharge

Rapid changes in river discharge due to flow augmentation during spring melt or fall were observed to be related to an increase or a reduction in nutrient concentrations in the Grand River (Figure 6.10). Cummings (2014) reported that the spring melt accounts for a large percentage of nitrogen annual export as tile runoff from agricultural fields. Loomer and Cooke (2011) reported that spring melt might responsible for a fair amount of the nutrients supply in the Grand River.

The increase in nutrients was observed as a flushing effect (*piston-like flush*); whereas the diluting effect was observced as a reduction in concentration of conservative tracers (electrical conductivity and chloride). An increase in nutrients and a decrease in conservative tracers did or did not occur sequentially in the Grand River between 2010 and 2013. Samples collected during fall storms allowed an estimation of the effects of high discharge on some water quality parameters.

The pistonlike flush was observed as an increase in total suspended solids and DIN concentration on the rising limb of the hydrograph; whereas the dilution effect was observed as chloride decrease (Figure 6.7), interpreted as increased surface runoff (increase in N inputs) that dilutes the chloride existent in the water column (assuming that no important contribution of chloride is present in the surface runoff). It is important to highlight that October 20th was

sampled in the morning and the afternoon; thus the two values on the same day correspond to samples collected seven hours apart. After the hydrograph peaked, there was total or partial recovery to background levels. A similar dilution effect was observed in electrical conductivity (Table 6.10). Thus, short and sudden changes in the river discharge are able to exert changes in water quality parameters, sometimes leading to increases in nutrients concentration, other times promoting decrease of the concentrations. The timing of these events and its relative importance to the total N flux and the trophic status of the Grand River and Lake Erie deserves further investigation. These type of pulses have also been observed in pharmaceutical in a Finland river receiving WTP effluent (Vieno et al 2005).

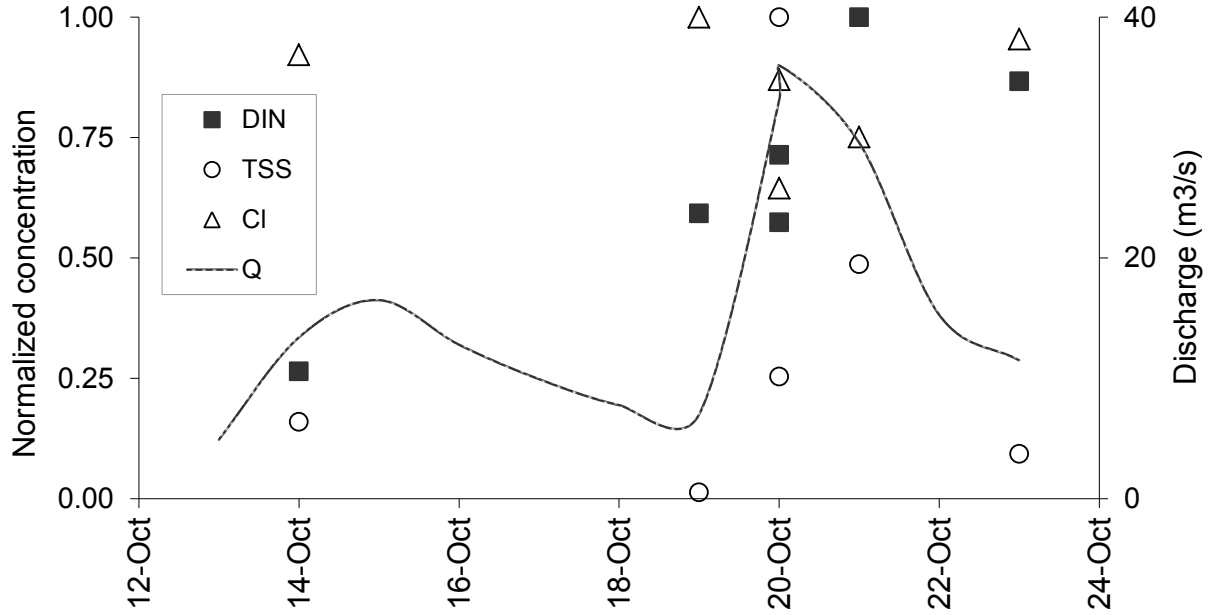


Figure 6.7 - The effects of pulse increase in discharge as a result of a fall storm in October 2011. The pistonlike flush was represented by an increase in nutrients and total suspended solids (rising limb), whereas dilution was observed as chloride decrease (falling limb). Dissolved inorganic nitrogen, total suspended solids and chloride are plotted as normalized concentration (normalized to the maximum concentration of each parameter during that period). Daily discharge data (m^3/s) from Water Survey Canada.

Table 6.10 - Changes in the Central Grand River water quality as a result of a pulse increase in discharge.

Seasonal event	Location	Distance (km)	Time period	Observed changes
Spring melt 2010	West Montrose	98	6 days	150% initial DIN increase, further decrease to almost initial concentration 50% decrease on Electrical Conductivity
	Blair	146	6 days	65% initial DIN increase, further decrease to almost initial concentration. 50% decrease on Electrical Conductivity. Chloride decreased to a third of the initial concentration.
	Brantford	188	6 days	No observed DIN increase Chloride decrease to a third of the initial concentration.
Fall storms October	West Montrose	98	5 days (Oct/2011)	30% increase ammonium, latter decrease to initial concentration. 60% increase DIN, DOC increase in one unit (1mgC/L) over a 9 days period). 1000% increase on TP and SRP at peak of hydrograph 50% dilution observed in chloride and sulphate.
			3 days (Oct/2012)	300% increase DIN (peak of the hydrograph)
	Blair	146	6 days	Doubled DIN concentration before the peak of the hydrograph. Oscillated between 4 - 4.2 mg DIN/L on the rising and falling limbs. 40% dilution observed on Electrical conductivity, stable around 0.49 --mS/cm on both rising and falling limb.
	Brantford	188	6 days	Slight increase in DIN (0.2 mgN/L) at peak of hydrograph. 50% dilution at peak of the hydrograph, diluted down to 63% on falling limb; observed on chloride

Agricultural vs Urban DIN contributions

The relative contribution of urban or agricultural NO_3^- can be estimated by subtracting the agricultural nitrate to the urban nitrate ($Nitrate_{urban} - Nitrate_{agricultural}$), Bridgeport representing the agricultural sources and Blair accounts for the NO_3^- generated in the urban area. In Figure 6.8, values above 0 represent dominant urban input.; values below 0 represent that agricultural inputs are greater than urban inputs at the location 5700 m downstream of the KTP. Cummings (2014) found that the tributaries in agricultural catchments in the northern Central Grand River (the Conestogo River and creeks) have higher nitrate concentrations than the Grand River, thus contributing more importantly than urban nitrate to the N inputs into the upper reach of the Central Grand River. The samples representing a fall storm in a dry year (see Figure 6.6, squares in fall, values below 0) provided supporting information about the important contribution of agricultural nitrate into the Central Grand River as a result of high discharge and increased surface runoff above the urban area.

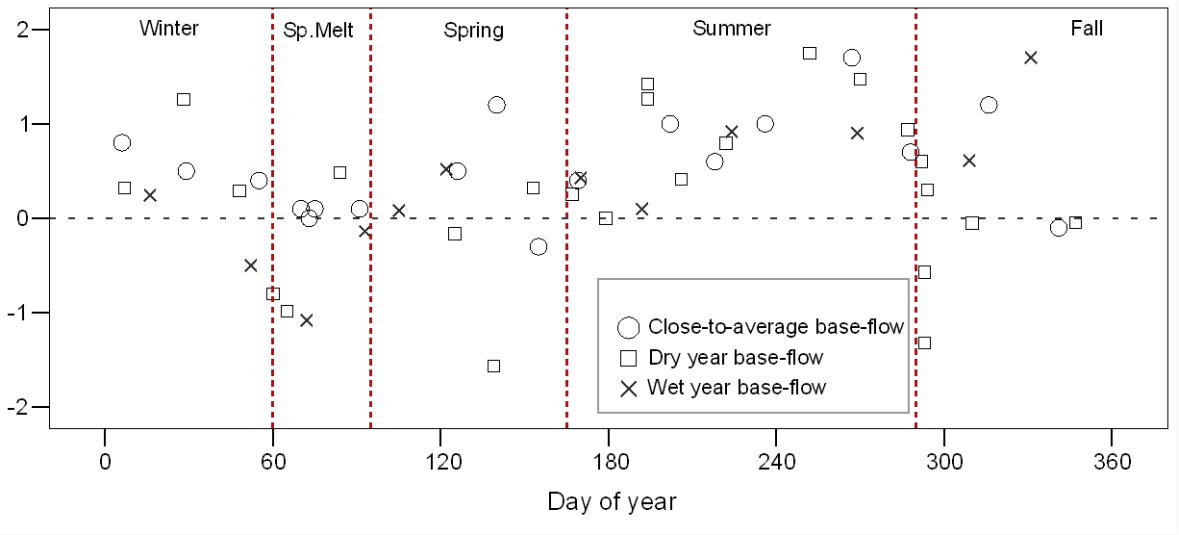


Figure 6.8 - Relative urban vs. agricultural nitrate contribution on different flow regimes, calculated as $Nitrate_{urban} - Nitrate_{agricultural}$, in mgN-$NO_3^-$/L. Agricultural impacts represented in the location Bridgeport (119 km from headwater); urban effects collected at the location Blair (145 km from headwaters). Values above 0 represent dominant urban inputs, whereas values below 0 represent dominant agricultural inputs. Seasonal demarcation based on normalized discharge.

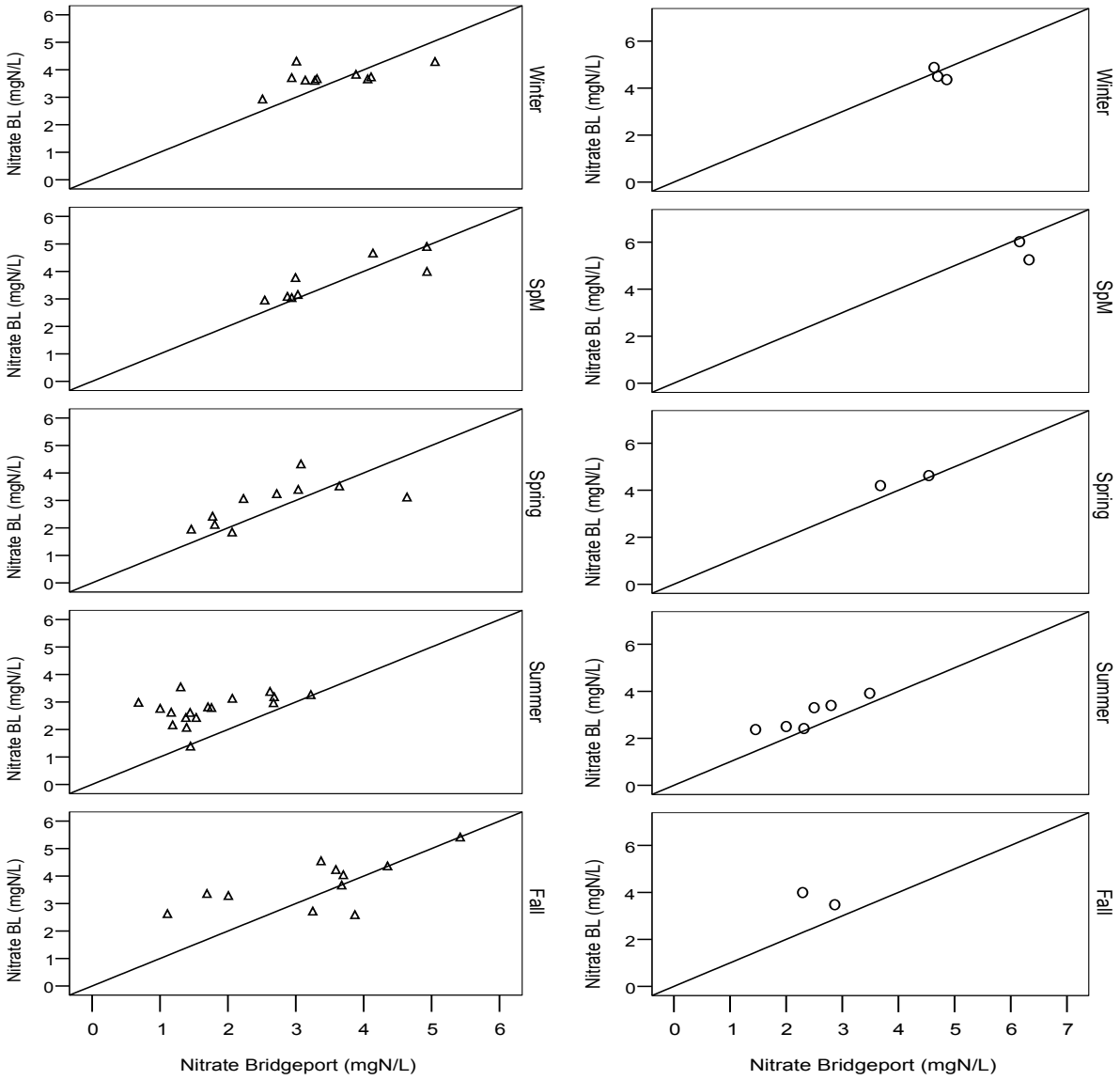


Figure 6.9 - Agricultural or urban-nitrate dominance in the Central Grand River before upgrades (2010 to 2012, *left column*) and after upgrades (2013 only, *right column*) by season. Values above the line represents urban-dominated nitrate inputs, values below the line represents agriculturally-dominated nitrate inputs. Concentrations expressed in mg $N\text{-}NO_3^-$/L. *SpM* stands for spring melt. See *seasonal demarcation* for details.

In general, when comparing the nitrate concentrations in years with different flow regimes, the urban NO_3^-contributions were observed to be more important than agricultural NO_3^-, particularly during summer. However, it is also possible that large part of the agricultural nitrate has been already assimilated; thus, not contributing as much as to the nitrate observed at

the location Blair. The relatively greater importance of agricultural nitrate during the low temperature season might be related to the above-discusse depressed biotic activity. The reduced amount of samples representing the after upgrades conditions (2013) do not allow to identify a long term trend. However, the urban NO_3^- still was the most important contribution to the NO_3^- downstream of the KWTP (samples above 1:1, line Figure 6.9).

Given that the DIN had an increasing trend as the Grand River flows southwards (Figure 6.10), it is considered that the Grand River is not assimilating all of the DIN generated in the agricultural and urban sub-catchments and some DIN is being exported downstream, representing large nitrogen inputs into the Lake Erie. The increase in N load after the KWTP upgrades (see Table 6.7) is considered to be not only the result of the upgrades in the WTP's, but also from the increase in population served and the treatment of the centrate (the highly concentrated residual water from the biosolids). With the upgrades completed so far, the WTP's are expected to serve the population at the current capacity until 2031 when additional oxygenation, increased residence time or anoxic bioreactors should be implemented (Wastewater Treatment Master Plan Report 2007).

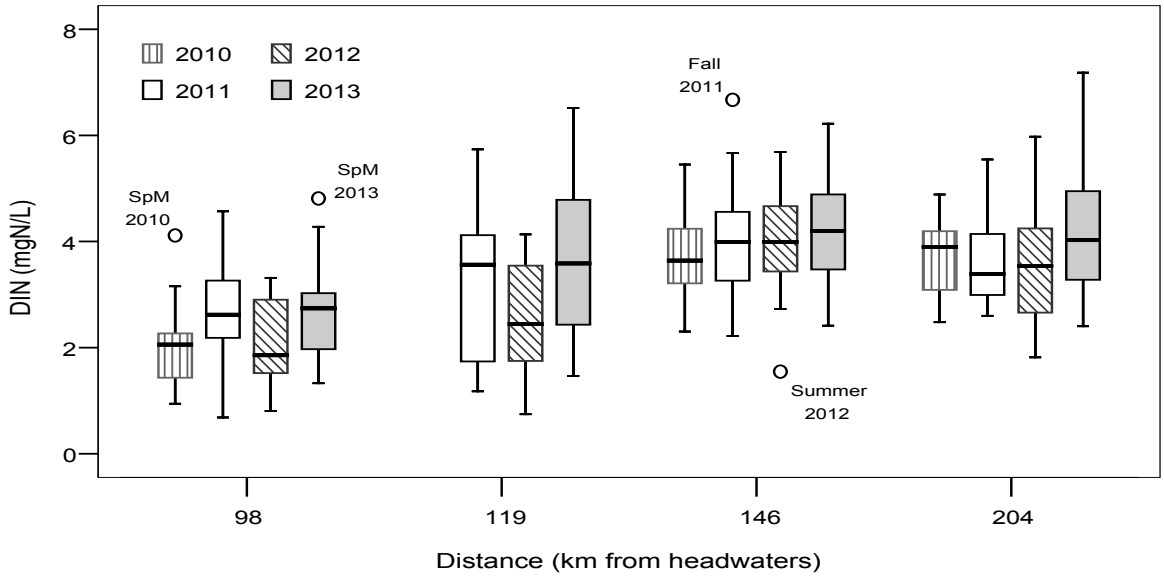


Figure 6.10 - Dissolved inorganic nitrogen (DIN in mg N/L) at four locations in the Central Grand River by year. 2010 to 2012 represent before upgrades, 2013 represents after upgrades conditions. 98 km-West Montrose, 119 km – Bridgeport, 146 km – Blair, 204 km – Brantford. Boxplots show the median (*black line*), interquartile range (*boxes*) and outliers (*circles*). Bars represent minimum and maximum.

NO_3^- represents 95 % (± 0.6%) of the DIN at the location West Montrose (98 km from headwaters) and 90% (±1.4%) at the rest of the locations downstream of the urban area. Monitoring nitrate is a common approach for assessing nitrogen in rivers, given that high nitrate concentrations and fluxes are typical for rivers with densely developed land and intensive agriculture (Ludwig *et al*. 2009).

Complementary to the inorganic nitrogen species, the dissolved organic nitrogen (DON) is an important component of the total dissolved nitrogen measured in the Grand River. The dissolved organic nitrogen (DON) was not studied extensively as the DIN in this research; however, DON accounted for an annual average of 24% (±12%) of the total soluble nitrogen (TN) measured in the Grand River. These measurements are in good agreement with previous reports in urban-agricultural landscapes (Neff et al. 2002, van Kessel *et al*. 2009).

Generally speaking, the DON resembles the DIN trends, increasing as the Grand River flows downstream. Comparing the sampling locations relative to their position to the KWTP effluent, the two upstream locations (West Montrose and Bridgeport) had different DON annual average concentrations, higher in 2011 than 2012 and 2013 (Tukey-Kramer HSD $q=2.43$, $\alpha=0.05$). DON concentrations at the locations downstream of the WTP's effluents were not different when comparing annual averages before and after upgrades. The samples from Brant Conservation Area (40 kilometres downstream of the KWTP) also had different DON annual average concentrations before and after upgrades (Student $t=2.03$, $\alpha=0.05$). The differences observed in DON concentrations are considered to be related increased surface runoff and the difference in the number of samples representing each condition (before n=239; after n=30). The different amount of samples beore and after upgrades prevents a comparison of the trend for a longer period. The DON is actively used by the planktonic and microbial community in nitrogen-poor environments (Kaushal and Lewis 2005), thus the DON possibly is not in high demand in the Grand River, where the DIN is abundant (N:P ratio ≥90 below the urban area).

Changes in the dissolved oxygen and phosphorus in the Central Grand River before and after the KWTP upgrades

Improvement in the dissolved oxygen in the Central Grand River was one of the main objectives of the KWTP upgrades. Before upgrades, he location closest to the KWTP (Blair) had hypoxic conditions during summer due to the large amount of ammonium released from the KWTP. As a result of the upgrades, the dissolved oxygen concentration at Blair was statistically higher after upgrades conditions (see Table 6.9). However, the DO concentration was still lower during the night than during the day. The ammonium chemical oxygen demand (COD) during summer before upgrades (July 2011) was estimated from 123 to 147 g COD s^{-1}, which was reduced down to 5.8 g COD s^{-1} after upgrades (August 2013; Region of Waterloo 2013); which represents a reducion in 96% of the COD. Not only the KWTP contributed to the low oxygen conditions at critical locations in summer, but also other WTP's discharging into the Grand River (Region of Waterloo wastewater treatment master plan report 2007); however, the impact of the KWTP was better assessed due to the continuous sampling at the location Blair. Assuming that hypoxia will not be common in the future, it is possible that preventing anoxic conditions might also hinder denitrification in the Grand River, potentially leading to higher-than-expected nitrate concentrations not only during winter, but also year-round. Despite the great change in the yearly average of total phosphorus, no statistical differences were found due to the large variance observed.

The existence of WTP's in the Grand River has been addressed as a detrimental factor for the ecosystem health (Mac Crimmon and Kelso 1970). Thus, the upgrades completed on the two largest WTP's of the Region of Waterloo are expected to have a positive effect on the health of the Grand River, the recreational uses and the drinking water quality. Water quality is particularly important during periods of low flow, particularly at Blair (due to low oxygen saturation) and Brantford (drinking water withdrawn from the Grand River). At the time that the Region of Waterloo wastewater treatment master plan report was released (2007), not enough data were available for concluding if some reaches in the Central Grand River will not meet the water quality objectives in the future due to the high nitrogen discharges from WTP's during low flow conditions. With the data here presented, it is inferred that the objective limits for water quality parameters in the Grand River (OMECC and Canadian Environmental Quality

Guidelines) will not be met in the future during dry years. For multi-year comparisons of physical-chemical and other chemical parameters, please refer to Appendix II

The entire Grand River before and after WTP's upgrades

After the WTP's upgrades (particularly the KWTP), the most conspicuous changes in the Central Grand River chemistry were lower ammonium and higher nitrate concentrations (Figure 6.11). By comparing data collected in September 2007 and September 2013 during the one-day whole river sampling campaign at the Grand River, it is possible to address some differences. These sampling events were chosen for seasonal comparison purposes and completeness of databases. However, the weather conditions for those years were different, 2007 was drier than 2013, thus the comparison of nitrate and ammonium concentrations should be done bearing in mind the differences in river discharge.

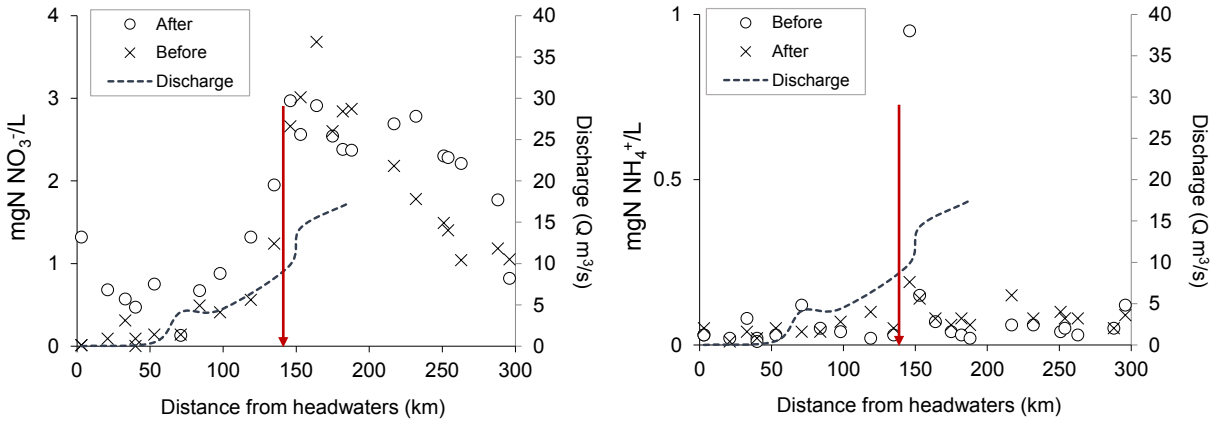


Figure 6.11 – Nitrate (*left*) and ammonium (*right*) concentration along the Grand River before (September 5th 2007) and after (September 10th 2013) upgrades of the Kitchener wastewater treatment plant. Effluent location marked by the red arrow. Samples were collected on September in each year. Discharge data available from Water Survey Canada for eight sampling stations only.

Given that the daily N discharge from the WTP's is relatively stable (Region of Waterloo 2014); the nitrogen discharged from the six WTP's above menitoned (see Table 6.7, 5.5 tonnes of nitrogen per day) is almost the same mass of nitrogen observed at Bridgeport (agricultural contributions, 4 tonnes N per day, daily average) in a dry year (2010). This addition of nitrogen means that, during dry years, the nitrogen from the urban area is the same as the agricultural nitrogen inputs, thus double the nitrogen downstream of the Region of Waterloo. Differently, these 5.5 tonnes of N per day from the WPT's in a wet year (2013), represents an increase of

25% of the agricultural nitrogen measured at Bridgeport (21 tonnes N per day, daily average). Therefore, the net effect of the the mass of N mobilized through the urban area needs to account for the mass of N originated in the agricultural region of the watershed (upstream the urban area) and the N assimilated in the river.

The dissolved oxygen saturation during the night (where photosynthetic oxygen evolution is at its minimum) remarkably improved after upgrades at the location 145 km from headwaters (Figure 6.12). The reduction in chloride and soluble phosphorus concentrations (Figure 6.13) appeared not to be a direct effect of the WTP's upgrades, rather an effect of dilution due to the above-average base flow in 2013. For instance, the chloride and SRP fluxes at the location Brant Conservation area (downstream of the urban area) were similar before (2026 kg Cl^-/d and 116 g SRP/d) and after upgrades (2032 kg Cl^-/d; 197 g SRP/d).

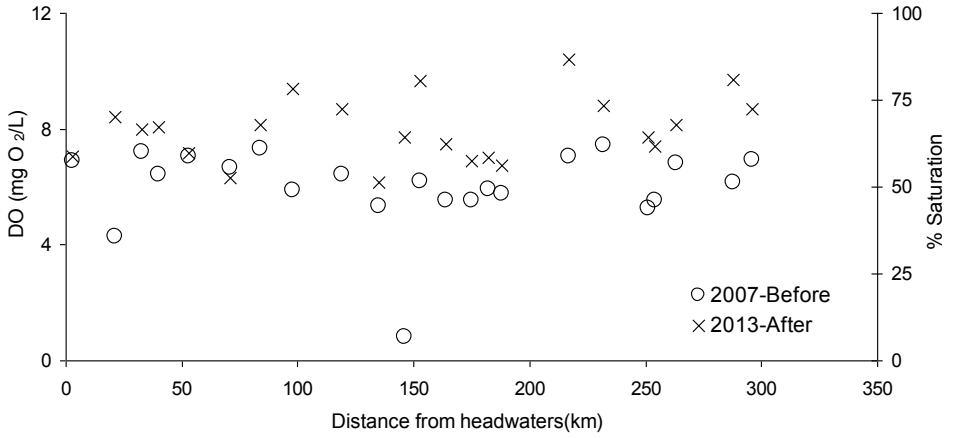


Figure 6.12 - Dissolved Oxygen concentration (mg O_2/L) and saturation (%) along the Grand River before (September 5th 2007) and after (September 10th 2013) Kitchener wastewater treatment plant upgrades. Samples were collected on September in each year, before solar dawn (4:00 to 7:00 AM). Saturation calculated from DOTABLES-USGS (http://water.usgs.gov/software/DOTABLES/).

Fluxes: the effect of changes in concentration and discharge

Changes in the river discharge entails changes in the fluxes of elements, not necessarily as a result of erosion, but as a result of human activities (Ludwig et al. 2009). Monitoring concentrations tracks the changes in water quality over time due to land use or anthropogenic impacts and collects information useful in planning nutrients management in agricultural and urban watersheds (Provincial Water Quality Monitoring Network). Concentration is a parameter used as a descriptor of the overall ecosystem health but largely influenced by

weather conditions. Fluxes and nutrient exports are particularly important when producing nutrient balances at watershed scales and are relevant for downstream receiving water bodies to address best management practices and geochemical budgets (Ludwig et al. 2009).

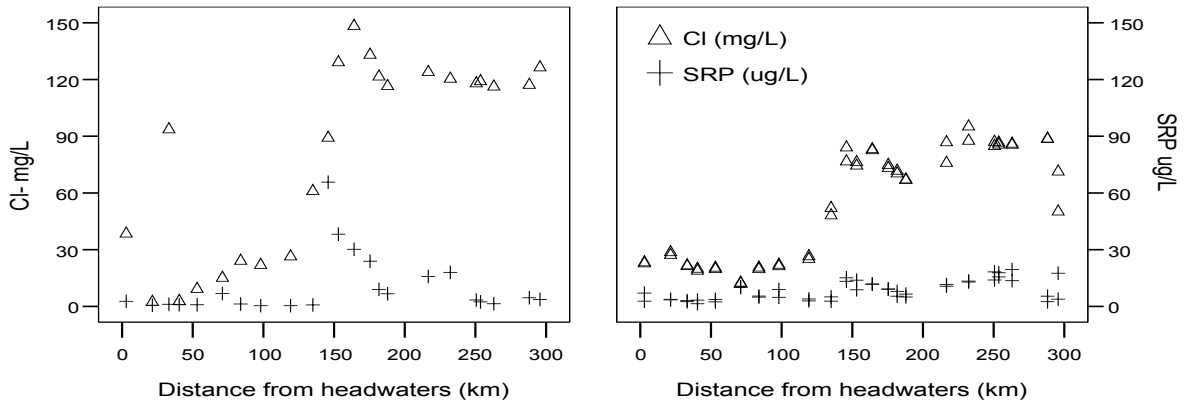


Figure 6.13 - Chloride (Cl^- in mg L^{-1}) and soluble reactive phosphorus (SRP in µg L^{-1}) concentration along the Grand River before (September 5th 2007, left panel) and after (September 10th 2013, right panel) Kitchener wastewater treatment plant upgrades.

The DIN flux estimated for the urban area of the Central Grand River was different between 201 and 2013(ANOVA F = 5.07, p = 0.02, df=101), higher in 2013 as a result the different river discharge). It was also different by location (ANOVA F = 9.21, p = 0.0002, df=101), commonly double in BCA (average = 24 tonnes DIN/d in 2010 and 49 tonnes DIN/d in 2013) that in Blair (10.8 tonnes DIN/day in 2010 and23.5 tonnes DIN/day in 2013). 2010 was a warm year with close-to-average base flow and moderated variability in DIN daily flux, showing a single, clear peak during spring melt. On the other hand, 2013 was a very wet year with above-average base flow, thus throughout the year there were observed large oscillations in DIN fluxes (Figure 6.14).

The spring melt event (March-April) is particularly important due to the large input of DIN into the central Grand River. The spring melt event in 2010 was clearly the most important season for N inputs into the central Grand River. However, in 2013, the peak observed at the agricultural area (98 km) and the one corresponding to the urban area (146 km) are clearly distinguishable; which means that the agriculturally-derived N might not be as important as the urban inputs during this high flow event. Additionally, the N entered into the Grand River

between West Montrose and Blair should be added to the N released by the WTP's; leading to the peaks observed at the urban area (146km). The increase in N observed below the urban area (204 km) is assumed to be the cumulative effect of all agricultural and urban inputs, in addition to the tributaries Speed and Nith Rivers and groundwater discharges.

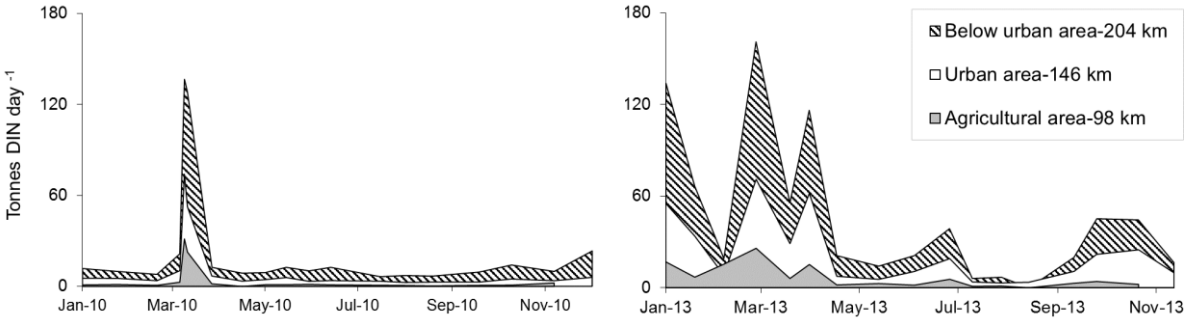


Figure 6.14 - Dissolved inorganic nitrogen fluxes (tonnes DIN per day) in three sections of the Central Grand River in 2010 (*left panel*) and 2013 (*right panel*). Agricultural area - West Montrose (02GA034); Urban Area - Blair (02GA048); Below urban area - Brantford (02GB001). Discharge data obtained from Water Survey Canada and GRCA. Distance in kilometres from headwaters.

Nitrogen loads are expected to increase as the population served by the WTP's increases, or if additional agricultural nitrogen is being added to the water courses. In the Grand River watershed, agricultural tributaries upstream of the urban area (*i.e*. Conestogo River and creeks) contribute to as much as 60 kg nitrogen per day during high flow season (March-April; Cummings 2014). Therefore, the nitrogen observed downstream of the Region of Waterloo is the the result of both agricultural non-point sources and urban point sources. Wet years (above-average year discharge) would likely have high fluxes at critical dates (such as spring melt and high precipitation events); whereas dry years (below-average year discharge) would have high nitrate concentrations punctually, especially below the urban area due to low base-flow.

Comparing concentrations without considering the river discharge is challenging and could be misleading due to the differential dilution of the nutrients and solutes transported across the watershed by year. High discharge was not always associated with high DIN concentrations in the Grand River. In fact, at three selected locations at the Central Grand River, high DIN concentrations were observed with moderately increased in flow (Figure 6.15). From these

comparison, it was observed that the Grand River had high nitrogen concentration regardless the river discharge observed. Additionally, high fluxes are also expected due to the large volume of water moving across the Grand River catchment.

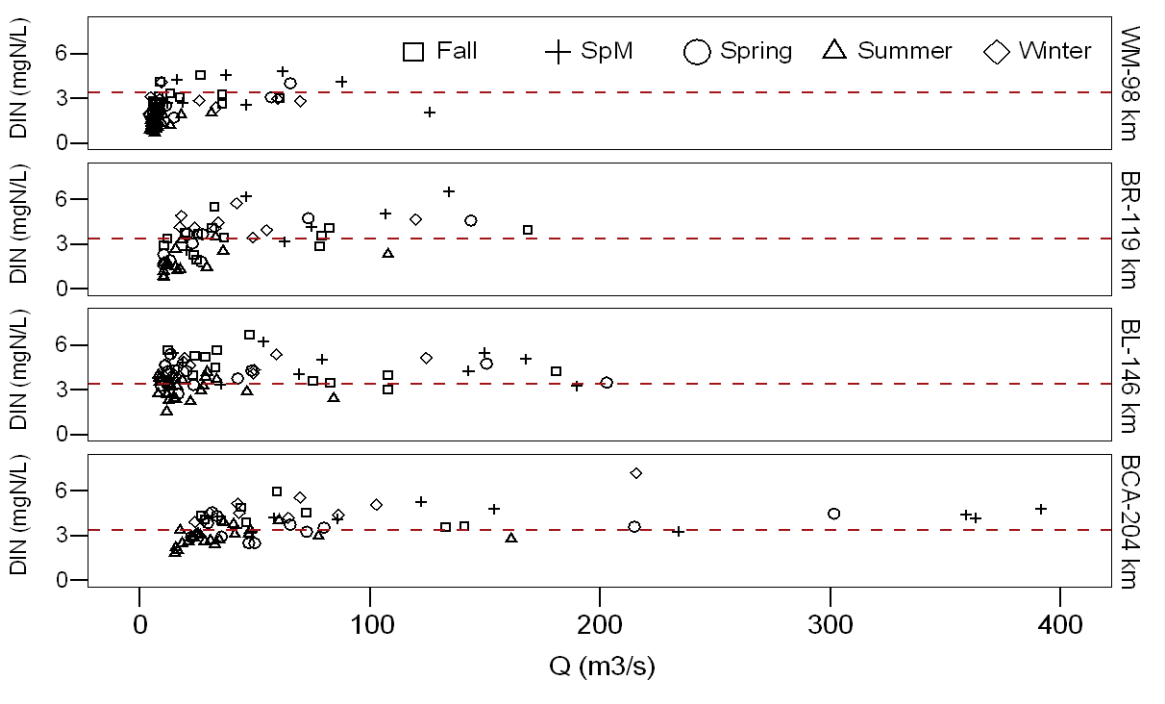


Figure 6.15 - Dissolved inorganic nitrogen (in mg N/L) and discharge (m^3/s) by season at four locations in the Central Grand River from 2010 to 2013. The vertical line represents the background nitrate concentration during winter in the Grand River above the Region of Waterloo (3.4 mgN-NO_3^-/L; Grand River Conservation Authority 2012). Distance from headwaters in kilometres.

Comparing concentrations among years is necessary to satisfy the regulatory framework and guidelines set by environmental authorities. The compliance with such guidelines ensures the proper functioning of the river as an ecosystem, and as the recipient and conveyor of treated effluent from urban areas. On the other hand, fluxes are necessary to calculate the mass of nutrients moving within the sub-catchments in order to correctly estimate nutrient balances within the Grand River watershed and exports to Lake Erie and the Great Lakes-St Lawrence river system. Accurate flux and export calculations require frequent water quality monitoring and discharge data (stage or flow velocity are also useful if the channel morphology is known); therefore, both monitoring strategies strength the capacity of doing better predictions and nutrient models; however, their design and smaplng strategy will be product-drived.

The human impact on rivers is greater for small-sized systems compared to major world rivers systems (Table 6.11) given that even small changes in the nutrient dynamics could affect small, shallow aquatic ecosystems. The Grand River watershed (6800 km^2) might reach large and fast changes with relatively small modifications in its nutrients regime due to its length and the small drainage area relative to its increasing population, leading to high levels of nutrients in slow-flowing sections of the river or increased macrophyte biomass. Dams are especially relevant when studying the biogeochemistry of an aquatic system due to the variety of impacts that damming causes to sediment movement (Yang et al. 2006), hydraulic and ecological niche modifications (Junk et al 1989, Biggs 1996), fish migration and imbalance of several nutrient cycles and ratios (Friedl and Wüest 2002).

Table 6.11 - Annual average nutrient concentrations in several anthropogenically-impacted rivers. Concentration in mg of N/C/P L^{-1}, respectively.

River	**NO_3^-**	**NH_4^+**	**TN**	**DOC**	**SRP**	**TP**
[1,2] Seine *(Europe)*	18.9	1		3.6		0.5
[1] Elbe *(Europe)*	3.9 *	1.4		5.3		0.4
[1] Chiang Jiang *(Asia)*	1.65	2.03			0.02 [§]	0.4
[1] Ganges-Brahmaputra-Meghna *(Asia)*	1.5 *	0.05			1.49	
[1] Murray-Darling *(Oceania)*	0.08	0.11		11.04	0.12	0.13
[1] Niger *(Africa)*	0.4 *	0.25				0.11
[1] Papaloapan *(South America)*	0.71	1.76	5.3		0.31	
[1] Mississipi *(North America)*	0.3 *	0.06		4.3		0.08
[1] St. Lawrence *(North America)*	0.19 *	0.08		2.6		0.02
[3] Grand River *(North America)*	1.9	0.33	2.42	5.47 [⌂]	0.09	0.32
[4] Grand River (North America)	*3.7*	*0.13*	*4.4*	*5.9*	*0.02*	*0.04*

[1] Water GEMS, year average values; [2] Meybeck 1993-NATO ASI Series I. Vol 4.; [3] PWQMN-2011, Station 16018403502-Dunnville, Ontario, 7 km from mouth of Grand River at Lake Erie; [4] *This research* at Brantford (204 km from headwaters) 2010-2013. * Nitrite+Nitrate; [§] Occasional data; [⌂] 2007-2013 Dunnville average.

CONCLUSIONS

The most important contribution of this research is that it provides a well-documented before-and-after case study of the effects of the DIN behaviour in an anthropogenically-impacted river

as a result of changes in the operation of WTP's. This research aims to provide with valuable and useful information that allows regulatory agencies and water managers (such as the Region of Waterloo and the Grand River Conservation Authority) to evaluate the effectiveness and the impacts of the upgrades completed on WTP's, to understand the changes in the nutrient status of the Central Grand River and to design the sampling strategy that better suit particular needs and optimize costs. This case study in the Grand River provides valuable information for the design and implementation of effective monitoring strategies and can be useful for regional municipalities or regulatory agencies planning to upgrade WTP's in areas with similar geographic and climatic conditions as the observed at the Central Grand River, Ontario.

The upgrades completed in the Kitchener wastewater treatment plant succeeded in reducing ammonium concentration in the effluent. However, the nitrate concentration in the Central Grand River downstream of the KTP effluent after upgrades in the fall of 2013 was above the Grand River nitrate target value of 3 mg $N\text{-}NO_3^-$/L). The COD was reduced 96%.

Differences in the DIN concentrations between seasons and between years are not only attributed to changes in the quality of the WTP's effluent, but also a result of upstream nitrate inputs from agricultural sources. Continuous nitrogen monitoring of both agricultural and urban sources is desirable to ensure that future N estimates are consistent and revised if necessary. The before-after approach used in this study of the Grand River allowed us to understand the dynamics of the DIN *in situ* as a result of changes in the operation of a wastewater treatment plant in an anthropogenically impacted river. The limited amount of data for the after upgrades conditions limits the interpretation; a monitoring period including years with above historical average and below historical average base flow would be desirable to capture the whole inter-annual variability and put in context the impact of the KTP upgrades on the Grand River.

The proposed normalized hydrograph is a practical approach to the interpretation of the weather variability observed in the Grand River and is potentially applicable to most urbanized rivers with flood-control structures. Intra-annual variations include seasonal effects such as changes in river discharge, water temperature and nutrient demand by crops. High ammonium and nitrate

concentrations concurrent with water temperatures lower than 15ºC were observed fall until mid-spring (over winter). Inter-annual variations are considered to be driven by river discharge.

Increases in river discharge caused dilution of nutrients and ions. In the future, when the population served by the WTP's increase, dry years (i.e below-historical average base flow) would have low dilution rate of the effluent in the river and likely an increase in nitrate concentrations to higher-than-expected levels. It will be important to establish sampling and monitoring protocols depending on the purposes and objectives of the diverse final users. High flow - high flux events are of special interests when evaluating nutrients export and producing nutrient balances at watershed scales. Monitoring concentrations is relevant for regulatory agencies dealing with water quality, environmental compliance limits and ecosystem health.

CHAPTER 7 -COMPREHENSIVE ESTIMATES OF DISSOLVED INORGANIC NITROGEN DYNAMICS IN THE GRAND RIVER: AN INTEGRATIVE APPROACH

This thesis has documented field measurements together with laboratory experiments in order to provide estimates based on the observed dissolved inorganic nitrogen dynamics in the central Grand River before, during and after upgrades in the Kitchener wastewater treatment plant. A brief summary of the main findings is provided in the following sections.

Summary of Kitchener wastewater treatment plant upgrades

Effluent from the KTP switched from high ammonium (13 to 26 mg N-NH_4^+/L) in before 2013, to high nitrite+nitrate (21-31 mgN-NO_3^-/L) starting early in 2013, as a result of upgrades. Although changes in KTP operation started in August 2012, oscillations in ammonium and nitrate concentrations were observed throughout the second half of 2012, until the new tanks were fully commissioned on January 2013 (Region of Waterloo 2014). Dissolved inorganic nitrogen (NO_2^-+NO_3^-+NH_4^+) loads entering the Grand River after the upgrades varied from 1707 to 2279 kg N/day (monthly mean). In the summer of 2013, ammonium plumes were no longer observed; nitrate comprised the majority of nitrogen inputs. Oxygen depletion conditions were reduced during summer low flow conditions and phosphorus and chloride loads were almost equal before and after the upgrades, but concentration in-river decreased due to dilution by high river discharge.

Summary of changes in total ammonia nitrogen

Ammonia volatilization estimates

The ammonia mass transfer coefficient k_{NH3} was calculated as 0.19 m/hr (or $5x10^{-5}$ m/s, corrected from k_{O2}). Thus, the ammonia flux representing volatilization in the Central Grand River between the KTP effluent and the sampling station Blair (5700 m) was calculated between 0.28 and 0.47 g N-NH_4^+ m^{-2} h^{-1}. Travel-time corrected data (average flow velocity of 0.3 m/s during low-flow conditions) allowed estimating the mass loss by distance. The N loss due to ammonia volatilization downstream of the KTP outflow, was estimated in 0.22 μg N-NH_4^+ L^{-1} m^{-1}, representing an average total N loss of 1.22 mg N-NH_4^+ L^{-1} by the time the Grand

River flows at Blair (5700 m downstream o the KTP effluent). Therefore, nitrogen loss by ammonia volatilization during summer low flow conditions represented around 50% of the total ammonium nitrogen discharged from the KTP. This estimate has been calculated with water samples collected inside the plume of nutrients for the entire reach of the Grand River between the KTP outflow and the location Blair at 5700 metres downstream. Due to variations in pH, temperature and ammonium concentrations, it was likely to expect variable N loss from ammonia volatilization among seasons before the KTP upgrades.

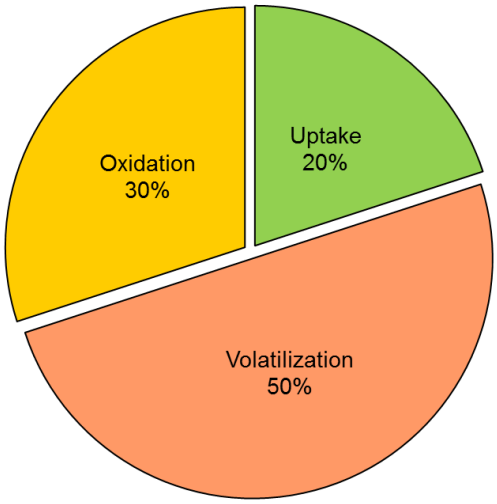
Uptake estimates in laboratory experiments

In laboratory conditions, estimated ammonium uptake rates by epilithon were between 4.8 and 7.2 mg $N\text{-}NH_4^+$ m^{-2} h^{-1}. Nitrate uptake rates were estimated in 0.8 to 0.9 $N\text{-}NO_3^-$ m^{-2} h^{-1}. The presence of light was a significantly different factor for the experimental assimilation of nitrate in epilithon. Assuming that the experimental uptake rates were similar in the field, the epilithic ammonium uptake rate in the Central Grand River downstream of the KTP was between 46 and 153 μm $N\text{-}NH_4^+$ m^{-2} h^{-1} , whereas the nitrate uptake rate was between 8 and 26 μm $N\text{-}NO_3^-$ m^{-2} h^{-1}. Considering the experimental results of ammonium uptake, at least 20% of the ammonium decrease could be attributed to uptake. This estimate is in good agreement with the calculations provided by Ogura *et al*. (2009) between 6 and 18% of ammonium decrease due to uptake; nonetheless, these uptake estimates do not take into account the uptake from macrophyte or other periphyton communities, therefore the uptake is likely underestimated when extrapolating uptake rates to the Grand River.

Summarizing the above-mentioned and assuming that epilithon *uptake* contributes with around *20%* of ammonium decrease and *volatilization* contributed with around *50%* of the total ammonium decrease during the daytime in summer low flow conditions before the upgrades on the KTP is showed in figure 7.1. Ammonia volatilization was estimated from field data, whereas uptake contribution (20% ammonium removal) is considered a conservative figure at the low end of the experimental results and previously published literature (see Chapter 2). However, it is possible that uptake would account for more than 20% of the ammonium decrease, either before or after upgrades. The percentage contribution presented in Figure 7.1

assumes that *microbial oxidation* (nitrification) account for approximately *30%* of ammonium reduction, the difference by subtraction.

After the upgrades in the KTP, the percentage contribution by process likely changed due to the lower mass of ammonium available. The contribution of ammonia volatilization was expected to be reduced (less than 50%) given that $NH_{3(aq)}$ is a function of the TAN concentration. It is assumed that the minimal uptake contribution is 20% and it is not possible to suggest a high end with the current information. The percentage contribution approach assumes that the rates were be the same before and after upgrades, which might not be the case after the upgrades. Due to the reduced mass of ammonium and the increased mass of nitrate, the rates of decrease (ammonia oxidation, assimilation and volatilization) cold be different; thus, yielding a different *proportional mass decrease* by process.



Assumptions of ammonium decrease

- *Uptake*: 20% of the ammonium decrease. Conservative figure considering the low end of experimental results and published literature.
- *Volatilization*: 50% of the ammonium decrease; estimated for samples collected inside the plume of ammonium during daytime (6:00 to 20:00) in summer low flow conditions before the upgrades on the KTP (2010-2012)
- *Oxidation*: 30% of the ammonium decrease. Difference by subtraction.

Figure 7.1 –Ammonium decrease contribution by process (in percentage) before upgrades during the daytime in summer, low flow conditions in the central Grand River downstream of the Kitchener wastewater treatment plant. Oxidation has been estimated from *Oxidation=100% - [Volatilization + Uptake].*

Ammonium preference

In order to properly estimate contribution of epilithon to nitrogen dynamics in experimental conditions, normalized uptake was calculated to produced net changes in total inorganic nitrogen (NO_2^-+NO_3^-+NH_4^+) as well as ammonium and nitrate individually (Chapter 2). Briefly, uptake in presence of both ammonium and nitrate varied among concentrations levels, but was

found to effectively consume nitrate at concentrations above 3 mgN-NO_3^- and 2.5 mg N-NH_4^+; yet preferring ammonium over nitrate, represented by the Rate of Preference Incorporation RPI=0.18) at a minimum ratio of 6.5 NH_4^+/NO_3^-.

Summary of changes in nitrate

Nitrate increase within the plume of nutrients leaving the KTP was calculated to be around 0.2 mgN-NO_3^- m^{-1} in 2010 and 2011(before upgrades), as a result of intense ammonium oxidation. After upgrades (2013), despite the fact that high nitrate was discharged from the KTP, the above-historical base flow observed in that year in the Grand River diluted the effluent, thus moderate nitrate concentration were observed downstream of the urban area. Predicted nitrate concentration in the 2031 for summer low flow conditions have been assumed to be 4 mgN-NO_3^-/L, and expected to be below 3.0 mg/L five km after the larger urban inputs (GRCA 2012). In spite of the modelled projection, samples collected on fall 2013 had 3.3 to 4 mg N-NO_3^-/L, with a maximum concentration of 6 mg N-NO_3^-/L in a sample collected in April 2013. Due to the high nitrate concentrations measured in-river after upgrades and the contribution of groundwater discharges with potential high nitrate concentration, the proposed increase in 1.1 mgN-NO_3^-/L will be likely surpassed, particularly in dry years (below-historical average base flow)

Depending on the nitrate concentration observed in a river, Miyajima *et al*. (2009) suggested two possible scenarios: *i)* conservative transport of nitrate, in which assimilation and denitrification is reduced and *ii)* dynamic balance between the inputs (nitrification) and the outputs (denitrification and assimilation). Neither of those scenarios are likely to occur in the Central Grand River in the 5700 m reach downstream the KTP before the upgrades. There was a slight increase in nitrate concentration observed downstream of the KTP effluent (see Chapter 4, Figure 4.14) and denitrification has been measured in this section of the Grand River (Rosamond 2013). However, there are some arguments in favor of a dynamic balance after the KTP upgrades, given the observed nitrate concentrations in summer downstream of the KTP: small changes in the nitrate concentration despite the fact that ammonium is no longer present in high concentration in the water column (no very intense ammonium oxidation) and so, nitrate is the only N source for assimilation.

Nitrate uptake

The Grand River below KTP could be alternatively a nitrate producer or nitrate consumer. Nitrate production was observed within the plume of nutrients during day hours, as a result of higher ammonium oxidation, due to in-river photosynthetic oxygen evolution. In the uptake experiment by epilithon, nitrate was modestly incorporated within the 48 hours period of the experiment, with a maximum uptake rate of 0.9 mg N m^{-2} hr^{-1}. In the treatments where nitrate was the only source of N, the assimilation was observed when the concentration in the experimental unit was 3.3 mgN-NO_3^-/L, and the initial N decreased between 5 and 11% in a period of 48 hours. Nitrate decrease in the presence of light was different given that the energy provided for nitrate reduction enhanced nitrate assimilation.

Kirchman and Wheeler (1998) measured assimilation between 4 and 14% of the nitrate available in the North Atlantic during algal blooms. A maximum of 27% N decrease due to nitrate uptake by phytoplankton has been reported by Deutsch *et al*. (2009) in the Elbe River. However, assimilation of up to 50% of the available nitrate has been reported for communities of heterotrophic bacteria (Middleburg and Nieuwenhuize 2000). More importantly, as nitrate concentration in the water column increases, uptake was observed to be stimulated (Mulholland et al. 2004). Therefore, given the complex (and unknown) taxonomic composition of the periphyton in the Central Grand River, nitrate uptake could be higher than the assumed in Figure 7.1, especially after upgrades of the KTP, given that is the main (if not only) N source.

Denitrification

Denitrification was not evaluated in this research. Rosamond *et al*. (2011) reported denitrification in the Grand River, representing nitrate removal between 2 and 11% of the nitrate in the Grand River. However, removal of 16% (Mulholland et al 2004) and 20% (Seitzinger *et al*. 2002) of the observed nitrate has been reported.

A conceptual model of ammonium and nitrate downstream of the Kitchener wastewater treatment plant

Concentrations above 5 mgN/L were observed starting in late fall 2012 and continued into the winter and spring of 2013. High DIN (particularly during winter) was also observed further

downstream, 20 to 42 km downstream of the KTP as a result of cumulative effluent from several wastewater treatment plants discharging into the Grand River. These increases in nitrate concentrations pose a concern for drinking water supply, which maximum acceptable concentration is 10 mg N/L, and potential harm for aquatic life particularly amphibians (OMAFRA - Environmental Impacts of Nitrogen use in agriculture Factsheet 2005). Finally, nitrite (NO_2^-) should be considered in studies where ammonium is present in relatively high concentrations (WTP's effluents or septic systems). In this study, nitrite represented as much as 25% of the DIN at some locations downstream of the KTP, similar spikes in nitrite could be expected in WTP's discharging effluent with high ammonium concentration.

Continuous nitrate addition (nitrification, surface runoff and groundwater discharge) and removal (assimilation and denitrification) occurs in the central Grand River downstream of the KTP. Summarizing the information presented throughout this research and previously published research, a conceptual model for ammonium and nitrate dynamics in the Central Grand River downstream the KTP before upgrades is shown in Figure 7.2; (upper panel) and after upgrades (lower panel). The most important change in the quality of the KTP effluent is the amount of ammonium and nitrate discharged before and after upgrades. Downstream of the effluent, the recently oxidized ammonium (*i.e* nitrate) add up to the upstream nitrate and the nitrate discharged from the KTP. These three nitrate fractions represent the expected nitrate downstream of the KTP.

According to the results obtained in this thesis, out of the total mass of ammonium discharged from the KTP (NH_4^+), around 20% is assimilated in plant cells, whereas 50% was estimated to be loss by volatilization (*Uptake+Volatilization* = -70%,). Thus, the remnant 30% ammonium is assumed to be nitrified (AO 30%, Figure 7.2). However, compared to the modeling results, the contribution of each process to the real ammonium decrease is likely somewhere between these calculations and the modeling results.

The expected nitrate increase has two processes of net removal from the water column in the Central Grand River. Denitrification as previously measured in the Grand River, removing around 11% of the observed nitrate in the river; and nitrate assimilation, with an approximated

nitrate removal of 5% of the observed nitrate (*refer to Chapters 2*). Therefore, denitrification and nitrate uptake has been estimated to represent approximately 15% removal of the observed nitrate. Figure 7.2 depicts the trade-off between ammonium and nitrate downsream of the KTP effluents. The bars represent the ammonium and nitrate concentration observed in the Grand River before and after upgrades; thus, the magnitude of the changes in concentration are characterized by the changes in the length of the bars.

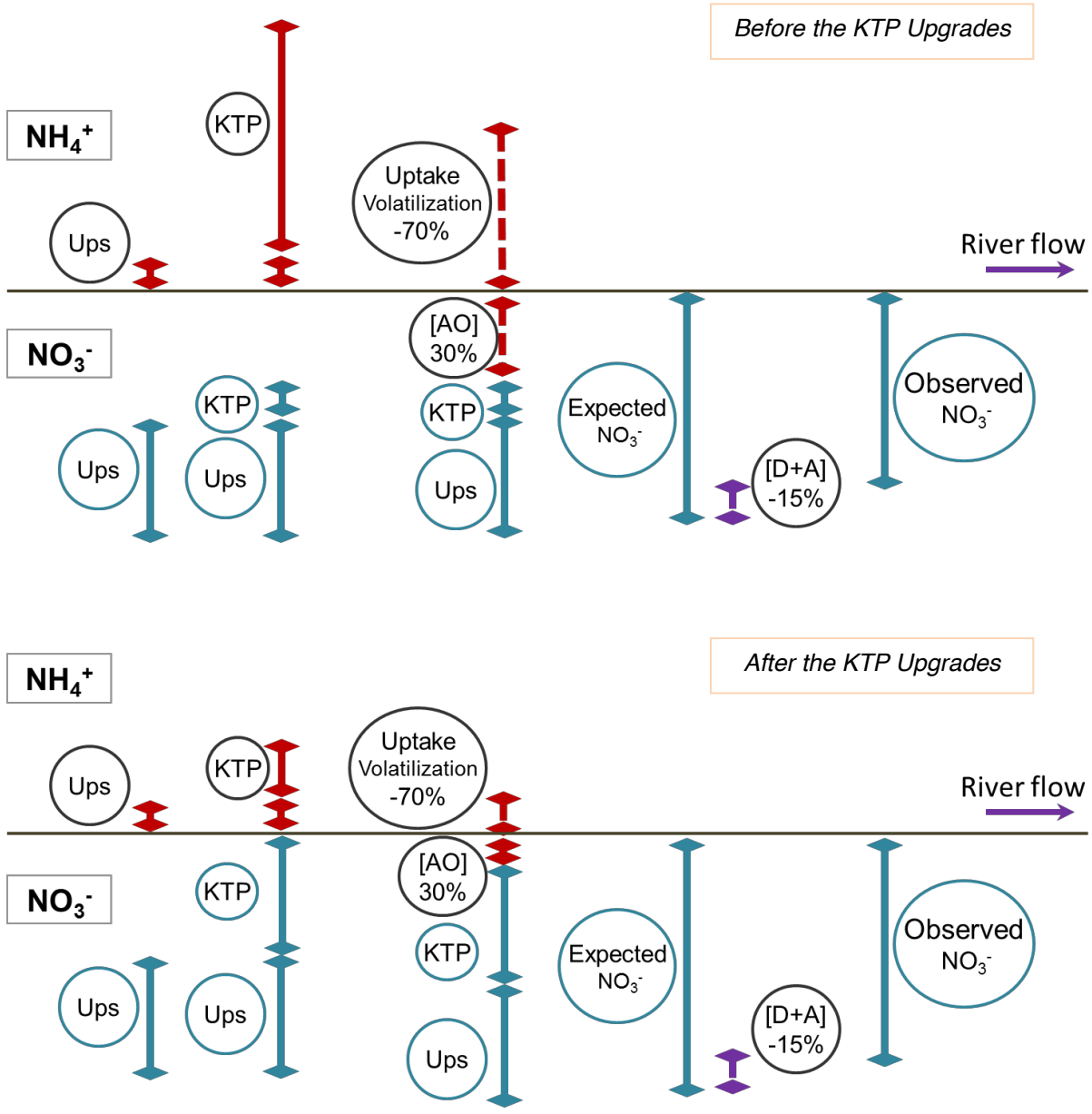


Figure 7.2 - Conceptual model of the dissolved inorganic nitrogen dynamics in the Grand River downstream of the Kitchener wastewater treatment plant before upgrades (*upper panel*) and after

upgrades (*lower panel*). *Ups* – Upstream of the effluent; *KTP*-Kitchener wastewater treatment plant; [*AO*]-Ammonium oxidation; [*D+A*]-Denitrification + NO_3^- assimilation.

During summer, low flow conditions downstream of the KTP, the expected ammonium decrease and nitrate increase between 2010 and 2013 at the location 5700 metres downstream of the KTP was estimated as follows:

Gross NH_4^+ decrease = avg NH_4^+ load effluent KTP – avg NH_4^+ flux at 5700 m
[AO] Ammonium oxidation = 0.3 x [avg NH_4^+ load effluent KTP]
Expected NO_3^- increase = avg NO_3^- flux upstream + avg NO_3^- flux effluent KTP + [AO]
[D+A] Denitrification + Assimilation = 0.15 x [Expected NO_3^- increase]
Net NO_3^- increase = Expected NO_3^- increase - [D+A]
Observed NO_3^- = NO_3^- flux observed at 5700 m

The Table 7.1 shows the results of these calculations. *Load* represents N mass added into the Grand River by the KTP and *flux* refers to the N mass passing a sampling location (in this case, 5700 m downstream of the KTP effluent). Loads and fluxes are expressed in kilograms of nitrogen per day. The good agreement between the expected and the observed nitrate flux supports the percentage contribution proposed for each of the processes downstream the KTP effluent with the data collected during summer low flow before, during and after upgrades of the KTP (Figure 7.3).

Table 7.1 - Calculated and observed nitrate increases (in kg N d^{-1}) downstream of the Kitchener wastewater treatment in summer low flow condition before (2010-2011), during (2012) and after upgrades (2013). Data for the KTP effluent was collected and provided by the Region of Waterloo. § Single sampling event, August 29th 2013.

Year	Gross NH_4^+ decrease	Ammonia Oxidation	Expected NO_3^- increase	Denitrif + Assim [15%]	Calculated net NO_3^- increase	Observed NO_3^- ± 1 s.d.
2010	2074	682	3744	562	3183	2962 ± 263
2011	1551	526	3386	508	2878	2722 ± 399
2012	1288	423	2686	403	2283	2032 ± 363
2013	10	3	5069	760	4308	3538 §

Given that a flat 15% has been assigned as nitrogen removal by denitrification and nitrate assimilation, the calculated values here presented could have been overestimated or

underestimated. The intra-annual variability is not included in these estimates given that the data used to produce these estimates represent water samples collected on one-day sampling campaigns as an example of the summer, low flow conditions in the Central Grand River before, during and after upgrades of the Kitchener wastewater treatment plant.

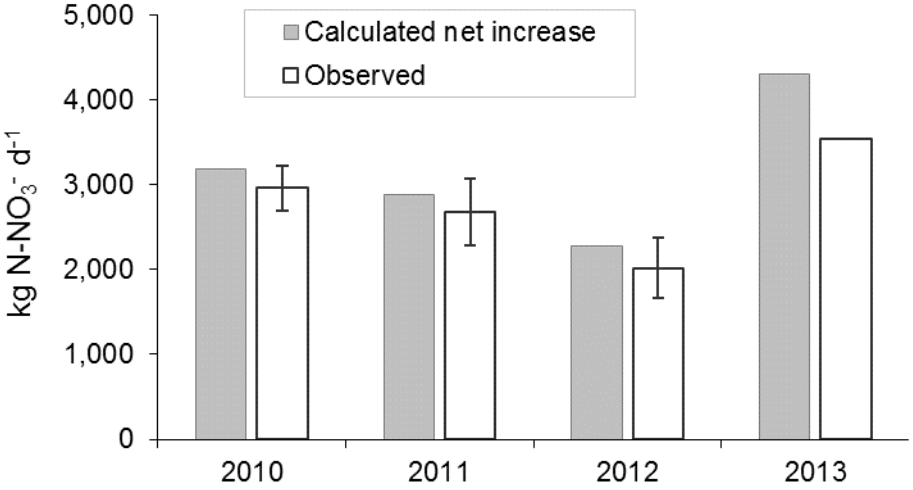


Figure 7.3 - Calculated net nitrate increase and observed nitrate fluxes (kg $N\text{-}NO_3^-$ d^{-1}) at the location 5700 m downstream of the Kitchener wastewater treatment plant during sampling campaing in summer low flow conditions after upgrades. One sample collected in 2013. Error bars ±1 s.d.

Vis *et al*. (2007) reported that changes in river discharge together with irregularities in the morphology of the water channel increases error in the calculated water volume, surface area and water retention times, especially when individual water masses converge into one water course. Thus, the accuracy of the estimated produced for the Central Grand River downstream of the KTP should always consider the daily variation of the volume effluent discharged from the KTP (see Figure 4.6, Chapter 4).

Water retention time and transient storage in the Central Grand River have important implications in nutrient cycling because it delays the mass transport in the surface or in the subsurface of the river (Runkel 2002). To my knowledge, transient storage has not been estimated in the Grand River. In wide and shallow rivers, a large fraction of the water column is in direct contact with the riverbed, thus water exchange with the hyporheic zone is likely to occur (Lautz and Siegel 2007). The large surface area for interaction with the Grand River bed might facilitate temporal storage of nutrients, leading to temporally nitrogen *disappearance*

from the water column, increasing the discrepancy between the estimated and the observed nitrate. In addition to transient storage at the hyporheic zone, the riparian zone of some streams and rivers could also store, retain or sequester dissolved inorganic nitrogen (Triska *et al*. 1994; Vought *et al*. (1994) possibly by nitrate uptake and denitrification. Such retention or storage could occur in the banks of the Grand River if the appropriate conditions are met.

Stable isotopes before and after the Kitchener wastewater treatment plant upgrades

Before upgrades, the decrease in ammonium concentration and the isotopic composition changed together as the river flowed south, varying according to the intensity of ammonia volatilization, nitrification and ammonium uptake. The upgrades of the KTP succeeded in reducing the concentration of ammonium in the effluent from more than 20 mg $N\text{-}NH_4^+$/L to 3.2 (± 2.4) mg $N\text{-}NH_4^+$/L. Consequently, the ammonium measured in the Grand River downstream of the KTP (corrected for dilution) decreased from ≈1.5 mg $N\text{-}NH_4^+$/L to less than 0.1 mg $N\text{-}NH_4^+$/L at the location 520 m from the effluent. Those changes in concentrations occurred simultaneously with an increase in the $\delta^{15}N_{NH_4^+}$ as a result of improved ammonia oxidation in the KTP, ammonia volatilization and ammonium uptake (see Figure 4.17, Chapter 4).

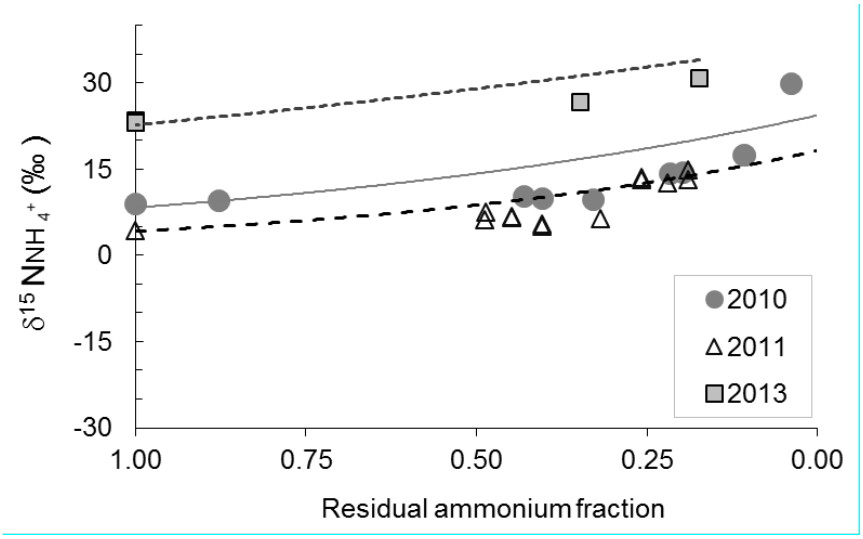


Figure 7.4 - Ammonium isotope delta ($\delta^{15}N_{NH_4^+}$) observed in the Central Grand River before (2010 and 2011) and after upgrades (2013) to the Kitchener wastewater treatment plant. Lines represent the model curves fit by the equation $\delta^{15}Nt = \varepsilon \ln f_xt$.

The observed $\delta^{15}N_{NH_4^+}$ before and after the KTP upgrades during summer low flow conditions of the year 2010, 2011 and 2013 is illustrated in Figure 7.4. The $\delta^{15}N_{NH_4^+}$ was fit to curves by the equation $\delta^{15}N_t = \varepsilon \ln f_t$. The best fit for the observed $\delta^{15}N_{NH_4^+}$ before upgrades (2010 and 2011) was for a fractionation factor ε =6 ‰ and a fractionation factor after upgrades (2013) ε =7 ‰ (Figure 7.4).

Ammonia volatilization, assimilation and oxidation removed the ammonium discharged from the KTP into the Central Grand River before upgrades in a similar fashion to the curves in Figure 7.5 (*left panel*). As discussed in Chapter 4, the model proposed that ammonium assimilation was the process contributing the most to the observed decrease in ammonium concentration; whereas the estimates obtained in this thesis suggested that ammonia volatilization was more important. However, there are two important differences between the model and the values estimated based on laboratory and field data. The estimated ammonia volatilization within the plume of ammonium observed downstream of the KTP effluent (the zone with the highest concentration of ammonium) and the ammonium assimilation was measured in experimental conditions only in epilithon, one biological component of the several present in the Grand River. Therefore, due to the uncertainties of the experimental results and the estimates presented in this thesis, it is possible that the curves representing ammonia volatilization, bacterial oxidation and uptake (Figure 7.5) could occur in any space of the shade area.

The nitrogen isotopic composition observed on the field is a combination of the isotopic fractionation factors of the three processes. The right panel of Figure 7.5 shows the observed $\delta^{15}N_{NH_4^+}$ together with the proposed individual $\delta^{15}N_{NH_4^+}$ measured for each process, assuming $\alpha_{volatilization}$= 1.019; $\alpha_{oxidation}$ =1.015 and α_{uptake} =1.010.

After upgrades, the ammonium concentration in the effluent was importantly reduced (0.8 - 5.4 mg $N\text{-}NH_4^+/L$) and the $\delta^{15}N_{NH_4^+}$ of the effluent was higher than 23‰. This ^{15}N-enrichment was the result of more efficient ammonium oxidation inside the KTP. As the ammonium became ^{15}N-enriched, the nitrate formed inside the KTP also reflected the ^{15}N-enrichment of the newly formed nitrate ($\delta^{15}N_{NO_3^-} \approx 13‰$).

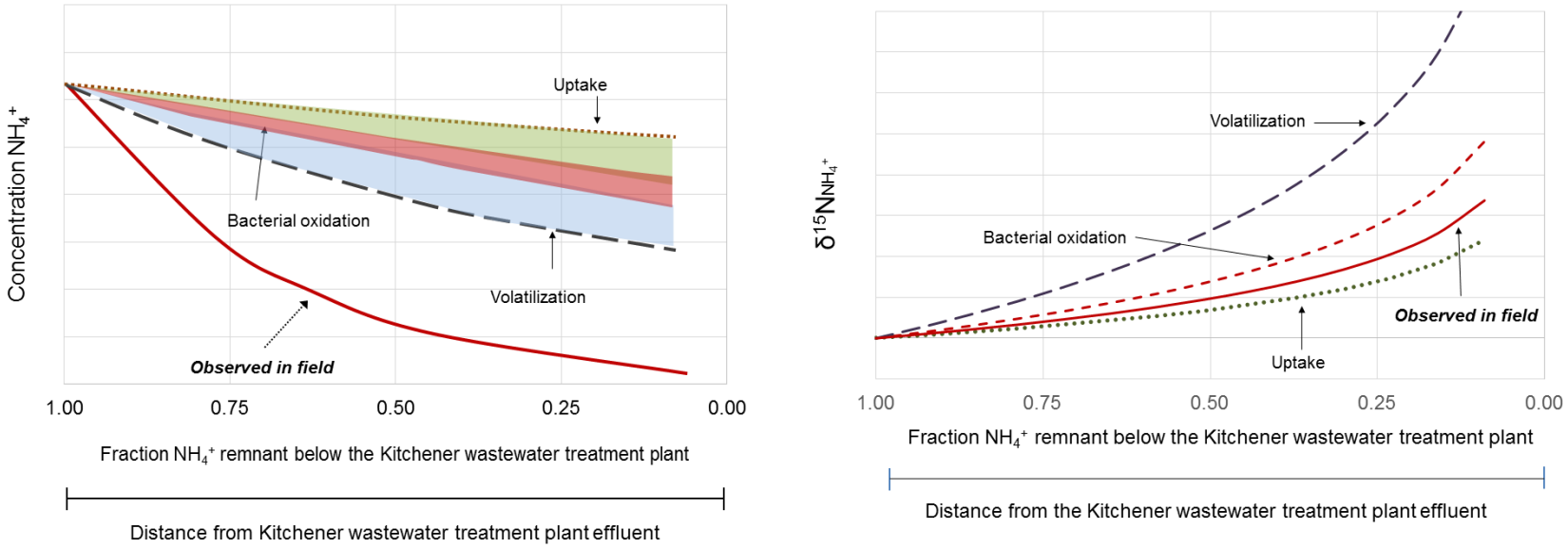


Figure 7.5 - Conceptual model of the decrease in ammonium concentration (*left panel*) and the $\delta^{15}N_{NH_4^+}$ (*right panel*) by different processes observed in the Central Grand River downstream of the Kitchener wastewater treatment plant in summer low flow conditions before upgrades (2010-2012). Ammonium concentrations for all analyses were corrected for dilution (using chloride as conservative tracer; see *Materials and Methods* in Chapter 4) Fractionation factors used in right panel: $\alpha_{volatilization}$= 1.019; $\alpha_{oxidation}$ =1.015 and α_{uptake} =1.010.

Final considerations

Is the Grand River functioning as a nutrients transporter or as a nitrogen transformer? This thesis proposes that the Grand River functions as both, nitrogen transporter and transformer alternatively in space and time. However, the transformative features are the most valuable environmental service that the Grand River provides, for it handles properly nutrients inputs from 30 wastewater treatment plants. Yet, as a result of the cumulative additions throughout its 300 kilometres of running waters, the Grand River is a transporter of nutrients into Lake Erie.

It could be argued that he plume of ammonium no longer exist when specific in-river parameters are back to base line; that is, the effect of a nutrient pulse has been assimilated by the river to such extent that background concentrations are recovered. Concentrations back to base line would be achieved if the river does not receive entering tributaries or external inputs (such as groundwater discharge) or any other type of dilution. Given the magnitude on the nitrogen inputs observed in the Grand River after receiving wastewater discharges from the two largest wastewater treatment plants (Waterloo and Kitchener), a return to base line was not achieved and likely will not be achieved in the future.

Although nitrogen and phosphorus are priority for water and wastewater management, concerns have been raised about increases in presence and concentrations of pharmaceuticals, estrogens, personal care products (Lishman et al. 2006) and chloride (from de-icing road salt, sewage and water softeners; Kelly *et al*. 2008). Water quality monitoring could also include emergent contaminants or substances that do not have stringent regulations. However, wastewater treatment plants have not being designed for controlling or reducing those substances; therefore, a different strategy for other compounds should be addressed a larger scale, such as the chemical of mutual concern initiative (Canada-US Great Lakes Water Quality Agreement, 2012).

Seasonal variations in river flow might also be important due to scouring and/or erosion of the riverbed, thus modifying the channel morphology. Water controlling structures release water according to reservoir levels or flow augmentation requirements. The release of water from dams together with rain interception increases the water discharge in the Grand River, promoting sediments movement and riverbed scouring in critical locations. Changes in the flow

regime would lead to changes in the travel velocity of solutes and particles. Investigating the hydraulic components that modify the chemical and biological processes occurring in the Grand River deserves further investigation.

Based on non-taxonomic procedures, it is suggested that the epilithic community in the Grand River downstream the Kitchener wastewater treatment plant might be predominantly comprised of Bacillarophyceae, Cyanobacteria and bacteria. The macrophyte community was not either taxonomically studied; yet, dominance by one or two species and its abundance changed when comparing before and after conditions. Although the light and flow conditions have not changed drastically, the dissolved nitrogen has; thus, there is a possibility of changes in diversity of the plankton and the macrophyte communities, which would require further investigation.

Large effort was put into the semi-intensive sampling of the central Grand River, however a large portion of the data collected correspond to summer and fall season (45 to 75% of the samples collected). The relative sampling intensity during these two seasons respond to logistic and safety reasons: collecting water samples at the desired location of a 5^{th} order river during high flow periods such as spring melt impose serious risk. Additionally, winter sampling faces the challenge of the Grand River being frozen for almost two months, which deter the sampling regularity under safe conditions. Similarly, the seasonal component allows properly designing and implementing sampling campaigns in critical dates or events, in order to better investigate the effect of seasonality onto the biogeochemistry of an urban river impacted by agriculture and sewage inputs.

GENERAL CONCLUSIONS

This research provides a well-documented before-and-after case study of the effects of the dissolved inorganic nitrogen dynamics in the anthropogenically-impacted Grand River as a result of changes in the operation of wastewater treatment plants and represents one of the few studies of the effects of wastewater treatment plant upgrades in a urban temperate river in North America. This thesis makes available field data and estimates that allows regulatory agencies and water managers (such as the Region of Waterloo and the Grand River Conservation Authority) to evaluate the effects of the upgrades completed on the Kitchener wastewater treatment plant and the changes in the dissolved inorganic nitrogen observed in the Central Grand River for a period of four years.

The most important change in the operation of the Kitchener wastewater treatment plant was the improved oxidation of ammonium; thus, the chemical oxygen demand of ammonium in the river declined and the dissolved oxygen in the central Grand River during summer, low flow conditions is no longer a concern for aquatic life. As a result of the improved ammonium oxidation, the nitrate concentration in the Central Grand River downstream of the KTP effluent after upgrades were higher-than-before upgrades several kilometers downstream of the effluent discharge.

For this research, the sampling efforts were focused on describing and explaining the influence of the Kitchener wastewater treatment plant effluent and its effects in the water quality of the Central Grand River during summer low flow conditions. Before upgrades in the Kitchener wastewater treatment plant, the plume of ammonium downstream of the effluent was found to reach more than 5700 metres during summer, low flow conditions. Additionally, this research provides an approach to estimate the length of a plume of ammonium in ungauged rivers receiving wastewater effluents. A customized, product-oriented monitoring strategy should be used according to the desired outcomes.

The processes contributing to the decrease in ammonium downstream of the Kitchener wastewater treatment plant were ammonia volatilization, ammonium uptake and ammonium

oxidation. The ammonia volatilization was estimated in 0.22 (± 0.4) μgN-NH_4^+/L per metre, (at a flow velocity of 0.3 m/s), representing a flux of between 0.1 and 0.3 mgN-NH_3 m^{-2} h^{-1}. Thus, ammonia volatilization was estimated to account for around 50% of the ammonium discharged by the Kitchener wastewater treatment plant into the Grand River. This is the first time that ammonia volatilization is estimated from a river receiving wastewater treatment plant effluent.

The ammonium uptake was evaluated on epilithon in experimental conditions, blocking nitrification by a chemical inhibition with acetylene. The epilithon established on the ceramic tiles used in the experiment, assimilated ammonium at velocities above 1 μm N-NH_4^+ h^{-1}. The estimated ammonium uptake rates were between 377 and 519 μm N m^{-2} h^{-1}. The nitrate uptake velocity was measured in 0.17 μm hr^{-1}, rates of 58 to 65 μmN m^{-2} h^{-1}. The experiments suggested that, in epilithon, ammonium is prefer over nitrate and that light had a significantly different effect on nitrate uptake. Due to the variable concentrations used in the experiments, epilithon uptake accounted for between 26% to 100% of the ammonium loss. The low end (approximately 20%) is considered a conservative figure for estimating ammonium uptake in the Grand River.

The results obtained after the upgrades are limited to one year; thus, future monitoring will help to complete the characterization of the after upgrades effects in the Central Grand River, especially during dry years. Intra-annual and inter-annual variations were considered and analysed, and it is expected that high nitrate concentrations will be observed downstream of the urban area of the Region of Waterloo during low base flow years (*i.e.*, dry years). Despite the fact that the effluent from the KWTP had an increased nitrate concentration, both nitrate losses and nitrate gains were observed in the Grand River. Differences in the DIN concentrations between seasons and between years were not only attributed to changes in the quality of the wastewater treatment plants effluent, but also a result of upstream nitrate inputs from agricultural sources.

The changes in ammonium and nitrate concentrations were also coupled to the change in its nitrogen isotopic composition. The overall isotopic fractionation due to ammonia volatilization in experimental conditions ($NH_4^+{}_{aq} \rightarrow NH_{3aq} \rightarrow NH_{3gas}$) was calculated as $\alpha_{volatilization}$=1.019

(±0.0024) at pH 8.5 and 1.030 (±0.0025) at pH 9.2. The equilibrium isotopic fractionation factor (α_{eq}) and the kinetic isotopic fractionation factor (α_{kin}) were estimated as α_{eq}=1.036 (±0.001) and α_{kin}= 1.050 (±0.003). The equilibrium and kinetic fractionation factors were obtained by an analytical method.

For ammonium assimilation, and based only on the observed changes in the nitrogen isotope delta of the submerged biomass of the Central Grand River (macrophytes and periphyton) before and after upgrades, the estimated discrimination against $^{15}NH_4^+$ was estimated in -5‰ before upgrades and -12‰ after upgrades ($\alpha_{assimilation}$ = 1.005 to 1.012). As a result of the changes observed between the substrate ($\delta^{15}NNH_4^+$) and the product ($\delta^{15}N_{macrophytes}$), it is proposed that the $\delta^{15}N_{TN}$ can be used as an environmental archive that incorporates the changes in the ammonium and nitrate isotopic composition of the effluent discharged into the Grand River. The $\delta^{15}N_{TN}$ of macrophytes and periphyton is, then, a complementary tool for monitoring the water quality and assessing changes in the water chemistry of rivers and streams receiving dischargeds from wastewater treatment plants

A comparison between the results presented in this thesis and a box-model that estimates the rates of ammonia volatilization, ammonia oxidation, ammonium assimilation and denitrification in rivers; indicates that the contribution of each process to the observed changes in ammonium and nitrate concentrations varied. However, in both cases, ammonium assimilation played a very important role removing a large part of the ammonium mass from the water column. The importance of biomass in removing ammonium was assumed to be the result of the great amount of biomass present in the Central Grand River downstream of the Kitchener wastewater treatment plant. The differences in the calculations between this research and the box model are that the box model estimated rates for all process simultaneously; whereas this thesis experimentally evaluated ammonium assimilation rates for epilithon only and that the ammonia volatilization rates could have been overestimated; the former representing short–term obsrevations in experimental conditions, the later for estimating equal rates of ammonia volatilization the Grand River in the centre and in the fringes of the plume of ammonium.

This case study in the Grand River provides valuable information for the design and implementation of effective monitoring strategies and can be useful for regional municipalities or regulatory agencies planning to upgrade WTP's in areas with similar geographic and climatic conditions as the observed at the Central Grand River, Ontario. Monitoring concentrations is relevant for regulatory agencies dealing with water quality, environmental compliance limits and ecosystem health. However, high flow - high flux events are of special interests when evaluating nutrients export and producing nutrient balances at watershed scales.

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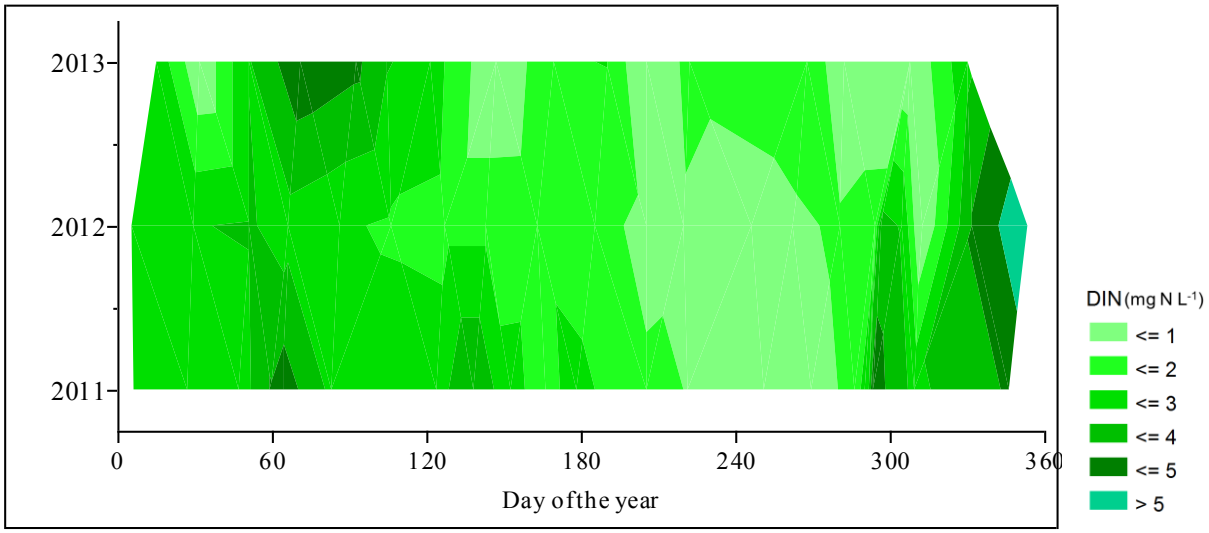
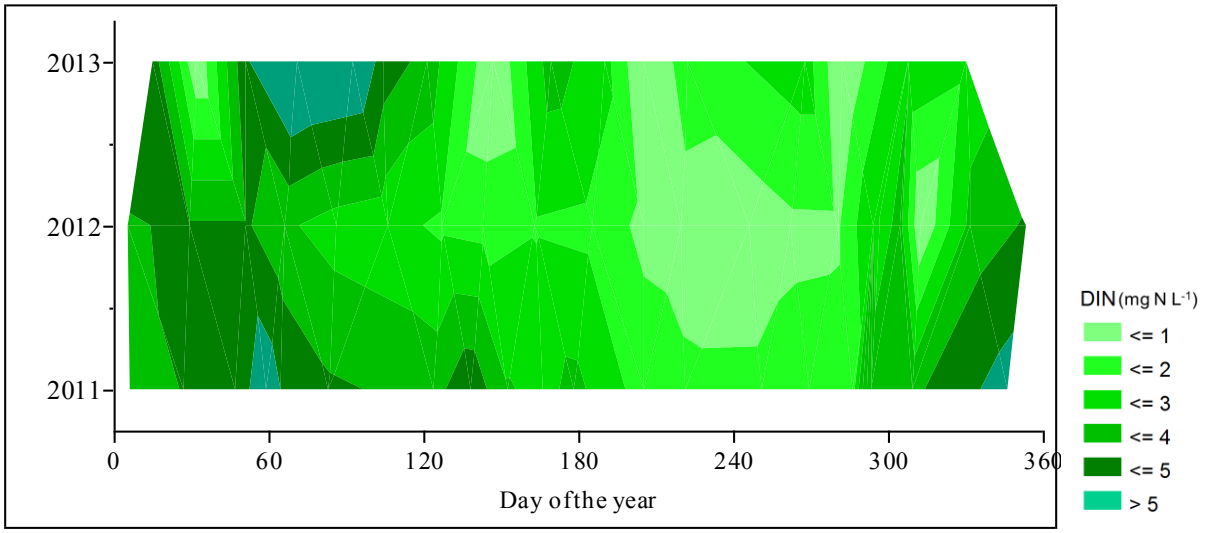
APPENDIX A

Dissolved inorganic nitrogen contour plots of the semi-intensive sampling sites from 2011 (before the KTP upgrades) to 2013 (after the KTP upgrades). Distance from headwaters in parenthesis. WM-West Montrose (98 km), BR-Bridgeport (119 km); VIC-Victoria St. (125km); BL-Blair (145 km); FB-Footbridge Rd. (158 km); GM-Glen Morris (164 km) and BCA-Brant Conservation Area (187 km). Contour plots were done with JMP 5.1.

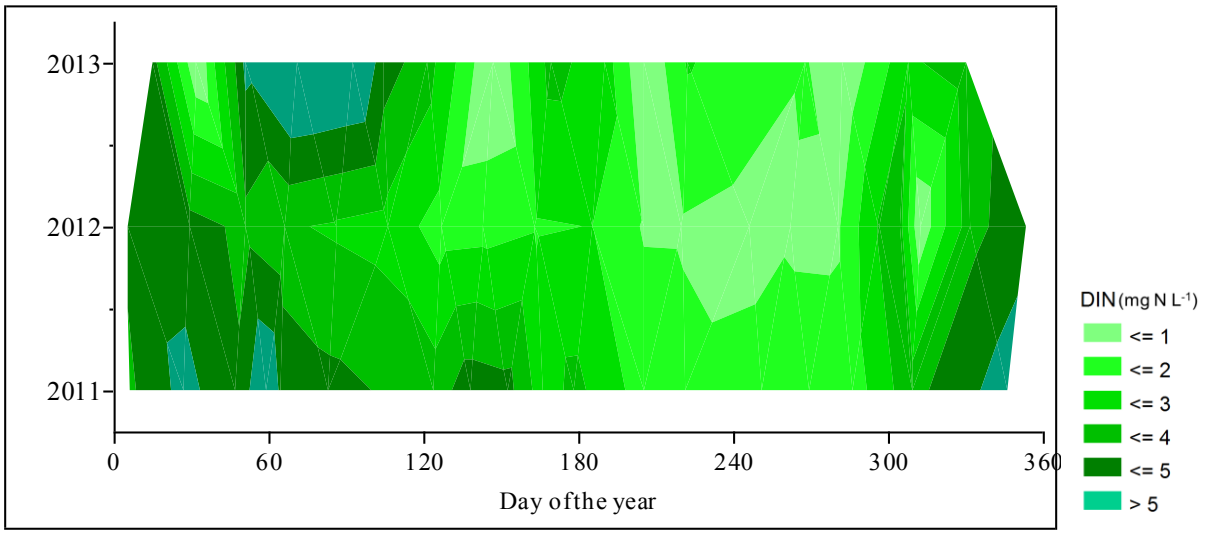
Location=WM



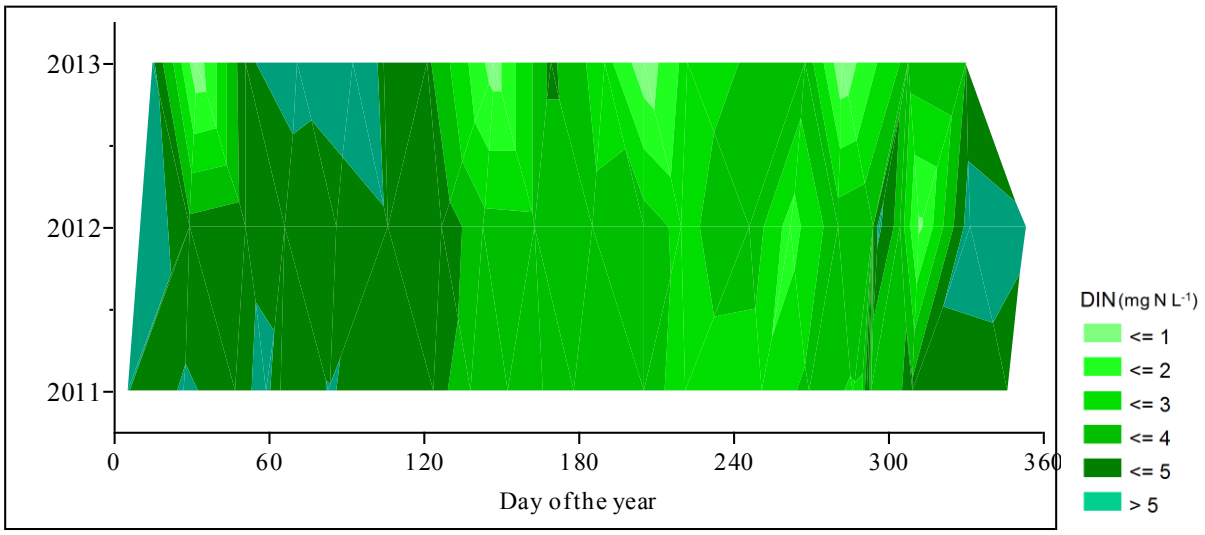
Location=BR



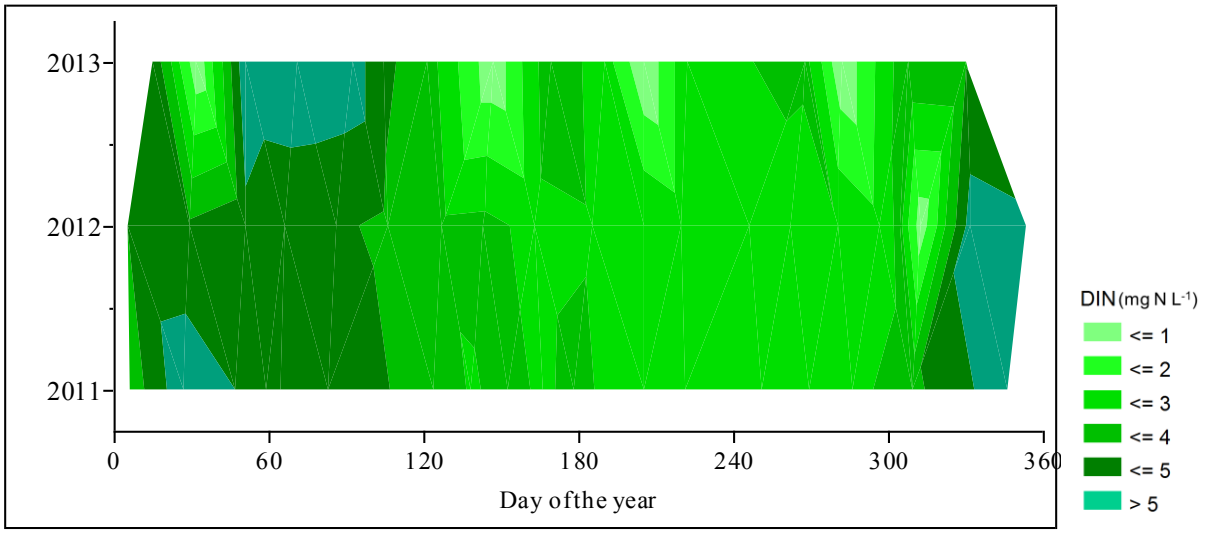
Location=VIC



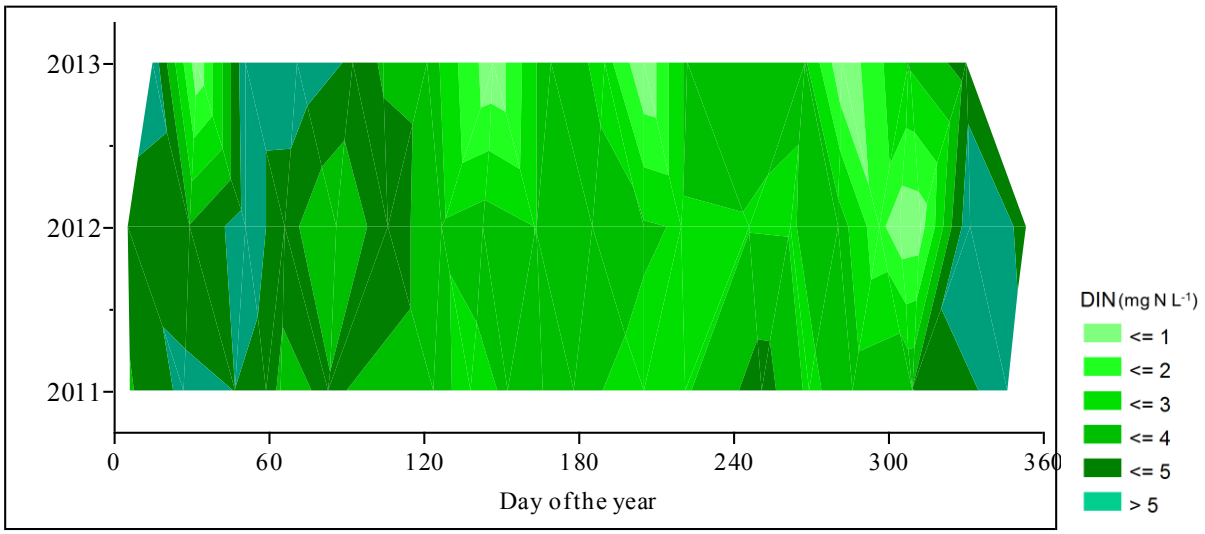
Location=BL



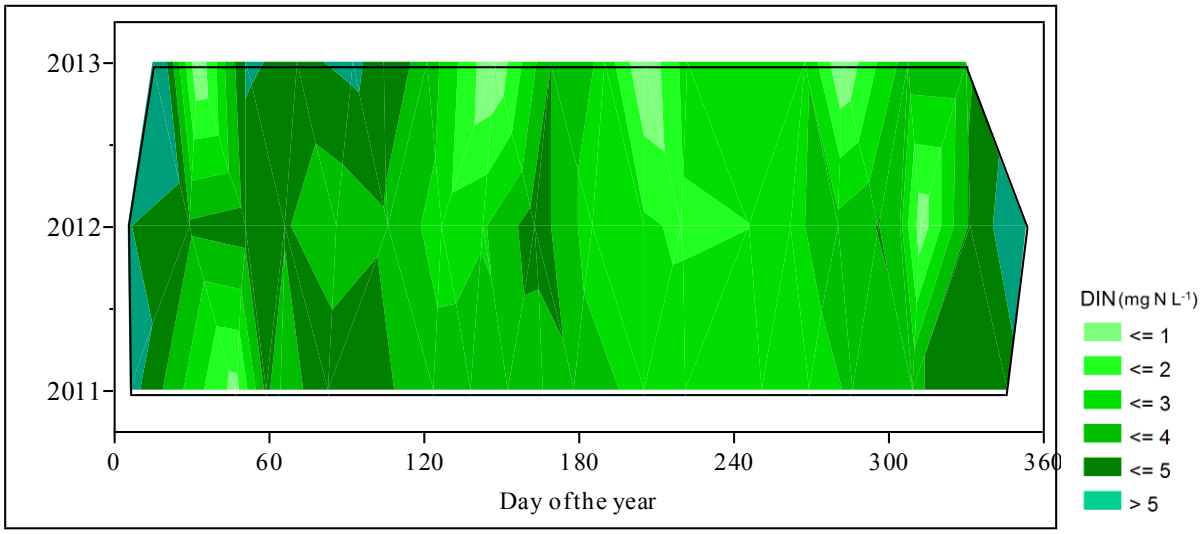
Location=FB



Location=GM



Location=BCA



APPENDIX B

Multi-year comparisons of physicochemical parameters (temperature, pH, electric conductivity and dissolved oxygen) of the semi-intensive sampling location in the Central Grand River from 2006 to 2013.

	Location		Temp (ºC)				pH			
YEAR	Km	Code	Mean	Median	Min	Max	Mean	Median	Min	Max
	98	WM	16.7	17.1	0.7	25.7	8.3	8.3	7.2	8.8
	119	BR	16.6	17.0	0.0	25.6	8.3	8.3	6.2	8.9
	125	VIC								
2006	146	BL	16.2	14.6	1.1	25.5	7.9	7.9	7.0	9.0
	158	FB								
	164	GM	17.3	15.2	4.9	26.2	8.3	8.2	6.7	9.0
	204	BCA								
	98	WM	13.1	17.6	0.0	25.4	7.9	8.1	6.1	8.7
	119	BR	10.3	12.3	0.2	25.3	8.2	8.2	7.5	8.9
	125	VIC								
2007	146	BL	10.6	5.2	0.2	25.5	7.9	8.0	6.6	8.4
	158	FB								
	164	GM	9.8	4.9	0.2	27.6	8.2	8.1	7.6	9.9
	204	BCA								
	98	WM	9.5	3.3	0.1	22.2	8.1	8.2	7.2	8.6
	119	BR	15.9	20.1	0.5	24.6	8.3	8.3	7.9	9.0
	125	VIC								
2008	146	BL	13.5	18.4	0.7	25.2	8.1	8.0	7.8	8.6
	158	FB								
	164	GM	10.1	6.6	0.8	22.6	8.1	8.1	7.8	8.4
	204	BCA	11.8	12.7	1.4	21.8	8.2	8.3	7.6	8.8
					-					
	98	WM	8.0	4.9	0.1	20.3	8.4	8.4	8.2	8.5
	119	BR	6.5	1.4	0.3	22.8	8.4	8.3	8.2	9.0
	125	VIC								
2009	146	BL	8.1	1.7	0.3	26.5	8.3	8.4	8.0	8.6
	158	FB								
	164	GM	7.5	2.7	0.2	26.5	8.5	8.5	8.1	9.1
	204	BCA	7.4	2.6	0.1	24.2	8.4	8.4	8.2	8.7

	Location		Temp (ºC)				pH			
YEAR	Km	Code	Mean	Median	Min	Max	Mean	Median	Min	Max
2010	98	WM	8.3	6.4	-0.3	21.0	8.0	8.0	7.8	8.5
	119	BR	9.9	8.0	-0.4	23.0	8.1	8.1	7.6	8.6
	125	VIC*	16.5	16.5	14.0	19.0	8.5	8.5	8.5	8.5
	146	BL	11.6	13.1	-0.3	23.6	8.0	8.0	7.7	8.9
	158	FB	12.9	12.4	-0.2	26.2	8.3	8.2	7.8	8.7
	164	GM	12.3	11.9	-0.1	29.2	8.3	8.3	7.8	8.9
	204	BCA	11.0	10.0	0.1	23.8	8.2	8.2	7.8	8.6
2011	98	WM	9.4	10.2	-0.4	22.3	8.1	8.1	7.7	8.6
	119	BR	11.3	10.7	0.2	25.3	8.1	8.1	7.6	8.9
	125	VIC	10.6	11.2	0.3	25.5	8.0	8.0	7.3	8.4
	146	BL	13.5	13.3	-0.3	26.2	7.9	7.9	7.4	8.7
	158	FB	12.3	13.1	0.1	26.5	8.1	8.0	7.6	8.6
	164	GM	11.9	13.3	0.2	25.6	8.1	8.0	7.7	8.6
	204	BCA	11.8	13.4	0.2	25.8	8.1	8.1	7.7	8.6
2012	98	WM	9.4	7.9	0.1	21.6	8.0	8.0	7.6	8.6
	119	BR	11.2	10.5	0.3	24.7	8.1	8.1	7.7	8.5
	125	VIC	11.1	9.7	0.6	26.5	8.2	8.2	7.7	8.7
	146	BL	11.7	10.9	1.0	25.1	7.8	8.2	1.7	8.5
	158	FB	12.5	11.9	1.0	26.1	8.4	8.4	8.1	8.8
	164	GM	12.2	11.2	0.9	25.1	8.3	8.3	7.9	8.6
	204	BCA	11.9	11.2	1.1	25.4	8.2	8.2	7.6	8.6
2013	98	WM	8.6	11.6	-0.1	19.3	8.0	8.1	7.5	8.5
	119	BR	9.2	9.6	0.1	19.9	8.1	8.3	7.8	8.4
	125	VIC	9.1	6.6	0.0	20.3	8.1	8.1	7.8	8.3
	146	BL	10.8	14.5	0.0	24.0	8.2	8.3	7.9	8.4
	158	FB	10.9	14.9	-0.1	21.9	8.2	8.2	8.1	8.4
	164	GM	10.5	10.8	-0.1	24.5	8.2	8.2	8.0	8.3
	204	BCA	11.0	14.5	0.4	22.1	8.0	8.2	7.2	8.3

* One-day sampling events.

	Location		E.C. (mS/cm)				D.O (mg/L)			
YEAR	Km	Code	Mean	Median	Min	Max	Mean	Median	Min	Max
	98	WM	455	455	390	572	9.4	8.6	5.0	16.9
	119	BR	489	494	436	654	9.5	9.0	4.6	16.9
	125	VIC*					13.1	13.2	13.0	13.3
2006	146	BL	693	699	510	811	7.1	7.7	1.0	12.7
	158	FB								
	164	GM	767	760	691	892	9.6	9.6	5.3	14.1
	204	BCA								
	98	WM	472	462	385	567	10.6	11.6	5.0	15.5
	119	BR	538	499	410	879	11.6	12.1	7.0	16.9
	125	VIC								
2007	146	BL	726	672	215	1120	9.2	9.0	0.8	18.2
	158	FB								
	164	GM	909	880	461	1800	12.0	12.7	4.4	17.5
	204	BCA								
	98	WM	516	424	305	1205	14.0	12.8	8.2	18.8
	119	BR	479	467	345	690	11.3	12.1	6.4	18.3
	125	VIC								
2008	146	BL	697	691	406	1285	10.3	11.1	3.8	18.6
	158	FB								
	164	GM	692	711	403	1304	13.3	13.8	6.3	18.0
	204	BCA	763	775	433	1618	11.8	12.5	6.4	16.9
	98	WM	340	358	173	579	10.8	10.6	7.2	14.8
	119	BR	346	345	168	618	11.3	11.7	7.4	14.3
	125	VIC								
2009	146	BL	494	538	195	734	11.0	11.5	6.9	14.1
	158	FB								
	164	GM	518	584	224	801	13.5	13.6	10.0	16.8
	204	BCA	480	466	216	768	12.6	13.5	9.0	15.6

* One-day sampling events.

	Location		E.C. (mS/cm)				D.O (mg/L)			
YEAR	Km	Code	Mean	Median	Min	Max	Mean	Median	Min	Max
	98	WM	502	498	234	686	10.2	10.7	6.5	13.7
	119	BR	541	519	375	747	9.6	9.4	6.4	13.0
	125	VIC*	817	817	817	817	10.7	10.7	9.2	12.2
2010	146	BL	711	658	447	1062	10.1	10.2	5.3	14.3
	158	FB	742	726	467	974	11.8	11.7	9.3	15.1
	164	GM	756	763	467	1020	12.3	13.0	9.1	16.2
	204	BCA	734	758	435	999	12.2	12.6	7.9	17.4
	98	WM	494	490	397	630	9.0	8.5	4.1	12.8
	119	BR	521	518	419	649	8.9	8.5	5.4	13.1
	125	VIC	561	537	426	735	8.9	9.3	5.2	12.8
2011	146	BL	686	704	453	1038	7.0	7.5	1.5	12.2
	158	FB	740	740	512	1074	10.1	10.0	5.6	13.6
	164	GM	782	761	568	1093	10.0	10.0	7.0	13.7
	204	BCA	740	733	506	1143	9.5	9.3	6.9	13.2
	98	WM	443	431	301	593	9.5	10.0	6.1	12.9
	119	BR	478	448	385	638	9.0	9.5	6.2	12.0
	125	VIC	511	482	387	752	9.2	10.0	4.7	12.6
2012	146	BL	681	675	468	1114	8.4	9.4	2.9	13.1
	158	FB	712	728	432	923	10.8	11.0	7.9	13.1
	164	GM	753	778	481	916	10.4	11.0	6.8	13.2
	204	BCA	751	772	554	888	10.5	10.5	6.1	14.3
	98	WM	451	470	229	537	10.7	10.2	7.7	16.7
	119	BR	522	513	441	682	10.7	11.0	5.4	14.5
	125	VIC	532	514	439	818	11.1	11.6	7.8	13.9
2013	146	BL	638	607	485	1196	11.0	11.1	7.8	13.9
	158	FB	625	583	486	1024	11.6	11.9	7.8	14.6
	164	GM	682	612	492	1308	11.7	12.0	8.2	14.8
	204	BCA	651	592	396	1115	11.5	11.8	8.0	14.0

* One-day sampling events.

APPENDIX C

Multi-year comparisons of ammonium, nitrate and total nitrogen of the semi-intensive sampling location in the Central Grand River from 2006 to 2013. * represents one-day sampling events

	Location		mg N-NH_4^+ / L				mg N-NO_3^- / L				mgN-TN / L			
YEAR	Km	Code	Mean	Median	Min	Max	Mean	Median	Min	Max	Mean	Median	Min	Max
	98	WM	0.056	0.031	0.011	0.515	1.5	1.2	0.5	4.7	3.7	3.3	3.1	2.9
	119	BR	0.036	0.028	<0.01	0.180	2.4	1.6	0.7	7.8	5.2	4.3	4.3	3.4
	125	VIC*	0.490	0.490	0.490	0.490	6.4	6.4	5.3	7.6	.	.	.	.
2006	146	BL	0.382	0.390	0.028	1.050	2.9	2.6	1.9	5.8	6.0	4.9	4.7	4.2
	158	FB												
	164	GM	0.075	0.071	<0.01	0.308	3.0	2.9	2.2	4.8	4.8	3.8	3.7	2.9
	204	BCA												
	98	WM	0.074	0.044	0.014	0.324	2.7	2.8	0.3	5.2	4.9	2.9	2.8	1.4
	119	BR	0.093	0.060	0.023	0.495	2.8	2.5	0.2	6.5	6.2	4.2	4.7	1.3
	125	VIC												
2007	146	BL	0.456	0.344	0.104	1.250	2.8	2.2	1.2	6.0	6.6	4.8	4.7	1.2
	158	FB												
	164	GM	0.541	0.422	0.048	1.440	4.0	4.0	2.3	5.8	6.9	5.2	4.9	3.7
	204	BCA												
	98	WM	0.056	0.038	0.021	0.116	2.8	1.7	0.5	6.7	7.7	3.7	3.2	1.0
	119	BR	0.055	0.038	0.015	0.227	2.2	1.4	0.6	9.0	10.0	4.7	4.8	1.4
	125	VIC												
2008	146	BL	0.344	0.314	0.131	0.818	3.3	2.5	1.6	7.2	8.2	5.4	5.4	3.5
	158	FB												
	164	GM	0.159	0.157	0.027	0.376	4.1	3.9	2.2	7.4	8.4	5.1	5.0	2.9
	204	BCA	0.083	0.043	0.017	0.479	3.7	3.3	2.2	6.1	7.6	4.7	4.3	2.8
	98	WM	0.065	0.040	0.014	0.141	1.7	1.9	0.5	2.6	7.8	2.8	2.6	1.0
	119	BR	0.152	0.059	<0.01	0.419	2.5	2.6	0.5	4.5	7.9	3.6	3.5	1.0
	125	VIC												
2009	146	BL	0.420	0.406	0.040	1.136	3.6	2.9	1.3	17.6	8.7	4.2	4.0	2.4
	158	FB												
	164	GM	0.309	0.300	0.028	0.856	4.2	3.1	2.6	19.7	7.9	4.2	4.3	2.8
	204	BCA	0.243	0.174	0.014	0.690	3.0	3.2	0.7	4.9	7.2	4.1	4.0	2.3

	Location		mg N-NH4+				mg N-NO3-				TN mgN /L			
YEAR	Km	Code	Mean	Median	Min	Max	Mean	Median	Min	Max	Mean	Median	Min	Max
	98	WM	0.070	0.071	<0.01	0.155	1.9	2.0	0.5	4.0	7.3	3.3	2.7	2.3
	119	BR	0.059	0.059	<0.01	0.217	2.7	2.7	1.0	4.9	6.3	3.7	3.6	2.5
	125	VIC*	0.634	0.634	0.587	0.680					4.6	4.6	4.6	4.6
2010	146	BL	0.449	0.211	<0.01	2.409	3.2	3.1	1.8	4.9	8.0	5.3	5.3	3.0
	158	FB	0.227	0.095	<0.01	1.045	3.5	3.3	2.4	5.0	6.9	4.8	4.6	3.8
	164	GM	0.250	0.120	<0.01	1.006	3.5	3.5	2.5	5.0	6.8	4.9	5.1	3.0
	204	BCA	0.190	0.050	<0.01	1.024	3.5	3.3	2.4	4.9	5.5	4.4	4.4	3.1
	98	WM	0.074	0.038	<0.01	0.456	2.5	2.4	0.6	4.5	6.4	3.4	3.0	1.7
	119	BR	0.152	0.017	<0.01	1.156	3.1	3.3	1.2	5.4	7.5	4.2	4.1	2.2
	125	VIC	0.187	0.052	<0.01	1.059	3.3	3.5	1.1	5.4	7.5	4.2	4.2	2.3
2011	146	BL	0.428	0.312	<0.01	1.472	3.3	3.1	1.9	5.4	9.0	5.3	5.0	3.7
	158	FB	0.231	0.051	<0.01	1.059	3.2	3.1	2.3	5.1	7.7	4.6	4.3	3.5
	164	GM	0.234	0.072	<0.01	0.983	3.5	3.4	2.5	4.9	7.5	5.0	4.7	3.6
	204	BCA	0.089	0.011	<0.01	0.541	3.5	3.4	2.6	5.5	7.1	4.7	4.6	3.6
	98	WM	0.099	0.038	<0.01	0.608	1.9	1.7	0.5	3.3	8.8	2.9	2.4	0.9
	119	BR	0.134	0.042	<0.01	1.569	2.2	1.8	0.4	4.1	6.7	3.1	2.5	0.9
	125	VIC	0.223	0.142	<0.01	1.424	2.2	1.7	0.5	4.5	7.1	3.3	2.6	1.1
2012	146	BL	0.792	0.661	0.100	2.540	3.0	2.9	1.3	4.5	7.5	4.7	4.6	2.3
	158	FB	0.327	0.254	0.015	0.885	3.1	2.9	2.0	4.9	7.5	4.4	3.9	2.2
	164	GM	0.311	0.234	<0.01	1.038	3.5	3.3	2.7	5.6	6.9	4.7	4.5	2.9
	204	BCA	0.191	0.145	<0.01	0.989	3.3	3.2	1.7	5.7	7.0	4.5	4.3	2.6
	98	WM	0.032	0.032	<0.01	0.061	2.5	2.6	0.1	4.8	5.1	2.9	2.6	1.6
	119	BR	0.038	0.014	<0.01	0.186	3.8	3.6	1.5	6.3	7.0	3.9	3.5	2.2
	125	VIC	0.073	0.022	<0.01	0.421	3.7	3.5	1.0	6.4	7.1	3.8	3.4	1.0
2013	146	BL	0.111	0.034	<0.01	0.494	4.0	4.0	2.4	6.0	6.5	4.3	4.1	3.0
	158	FB	0.124	0.045	<0.01	0.490	4.0	3.9	2.5	5.6	6.4	4.1	3.7	2.6
	164	GM	0.118	0.047	<0.01	0.476	4.0	3.7	2.6	5.4	6.3	4.0	3.8	2.4
	204	BCA	0.063	<0.01	<0.01	0.249	4.1	3.9	2.4	7.0	7.5	4.2	3.8	2.4